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ELIMINATION OF RADIOIODINATED ANTIRICKETTSIAL HOMOLOGOUS GAMMA GLOBULIN FROM NON-IMMUNE AND SPECIFICALLY IMMUNIZED RABBITS*

LUDWIK ANIGSTEIN, DOROTHY M. WHITNEY AND WILLIAM BARNES

The tracing of heterologous gamma globulin by radioisotopes has demonstrated the zone of activity of tissue antibodies in the injected host, the selective anatomical localization and the characteristic retention-time for various tissues (Pressman and Keighley, 1948; Pressman, 1949; Pressman and Sherman, 1951; Anigstein *et al.*, 1952; Anigstein, 1953). Whereas this phase of tissue immunology has greatly benefited from the use of isotopes as tracers, the technique has only recently been applied to the tracing of passively administered gamma globulin (Dixon *et al.*, 1952). The specific immunity of the host was found an important factor influencing the fate of injected heterologous gamma globulin. It was shown that specifically immunized rabbits eliminate bovine gamma globulin from the blood more rapidly than non-immune controls (Dixon *et al.*, 1950, 1951; Laws and Wright, 1952). This phenomenon was first observed by Glenny and Hopkins (1923) in their fundamental studies on the duration of passive immunity. In injecting equine diphtheria antitoxin into non-immune rabbits and into rabbits previously sensitized against horse serum, an accelerated loss in the antitoxin was established in the immune animals. The striking similarity of the three-phase elimination curves for the horse serum expressed in antitoxic units with the clearance curves for radioiodinated gamma globulin (Dixon *et al.*, 1951; Talmage *et al.*, 1951), is of considerable interest inasmuch as these phenomena were revealed independently by entirely different technical approaches.

The effect of iodination on antibody activity *in vitro* has been studied by Pressman and Sternberger (1950) who demonstrated

* From the Rickettsial Research Laboratory, University of Texas Medical Branch, Galveston, Texas. This work was supported by a contract (NR: 130–981) between the Office of Naval Research, Department of the Navy, and The University of Texas, and was presented at the 53rd Annual Meeting of the Society of American Bacteriologists, San Francisco, August 10–14, 1953.

that the introduction of 18 atoms per antibody (anti-ovalbumin) molecule still does not destroy its stability to undergo specific precipitation. The stability of the labeled rabbit antibody in guinea pigs was demonstrated by quantitative precipitin reaction by Melcher and Masouredis (1951). The findings of these authors indicate that the I 131 label of rabbit antibody does not exchange *in vivo* by an independent metabolism process.

The validity of radioiodination of gamma globulin for the determination of antibody life has gained additional weight by the work of Dixon, Talmage, Maurer and Deichmiller (1952) who determined the half-lives of homologous gamma globulin for various animal species and found that the values obtained by the isotope techniques agree well with the half-lives determined by immunochemical methods.

It is the purpose of the present study to trace the distribution and fate of radioiodinated antirickettsial homologous gamma globulin in the blood, tissues and urine of non-immune and of specificially sensitized rabbits. Inasmuch as only soluble antigens (mostly foreign proteins) were previously used by others and whereas microbial antigens have not received detailed consideration, it was felt desirable to include rickettsial organisms into the present study. Consequently, various series of rabbits were sensitized with the antigens of *Rickettsia rickettsii*, *R. prowazeki* or *R. mooseri*. The influence of the specifically immunized host on the disappearance rate and the half-life of the injected radioactive gamma globulin was then investigated.

Materials and Methods

Immunization of rabbits. Rabbits, mainly males, weighing about 3 kg. were immunized against Rocky Mountain spotted fever, epidemic and murine typhus by intravenous (0.1 to 0.2 ml.) and intramuscular (0.5 to 0.75 ml.) injections of commercial vaccines supplied by Lederle Laboratories Division. The antigenic constituents of these vaccines contain the soluble 'S' immunizing antigen in the rickettsia-free vaccine liquor in addition to the extracted suspension of killed rickettsial bodies (Chambers and Clawson, 1950). Usually 4 to 5 injections were given to a rabbit over a period of 20 days. Eight days after the last injection the rabbits were bled and the sera tested for complement fixing antibodies. The titers ranged from 1:160 for typhus antisera to 1:640 for spotted fever.

Preparation of the globulin fraction, radioiodination and dosage. In addition to the gamma globulin fractionated from normal and immune rabbit sera (RGG) and prepared in this laboratory, refined and concentrated hyper-

immune sera against Rocky Mountain spotted fever and epidemic typhus were obtained from the Lederle Laboratories Division by courtesy of Dr. Herald R. Cox. The globulin content of these sera was in the average 70 mg./cc. as compared with an average of 20 mg./cc. in our antisera. The globulin fractions of both series were analyzed electrophoretically by the Tissue Metabolism Laboratory under the directorship of Dr. W. W. Nowinski and showed a high degree of purity in gamma globulin. Series of nonimmune control rabbits and actively immunized rabbits were subsequently injected with tagged normal or antirickettsial gamma globulin (I*RGG).*

The separation of the globulin fraction from undiluted normal and immune sera was accomplished by the ammonium sulfate precipitation procedure according to Eisen and Pressman (1950). The dialysing system in concentration of 1.75 M ammonium sulfate in a cellophane bag was rocked overnight in a refrigerator. This was followed by rotary dialysis at about 5°C against 17 liters of 0.16 M saline, overnight or longer, until the dialysate was ammonium sulfate free. The precipitate was then lyophilized and the white crystalline fraction of each globulin sample was radioiodinated by the Abbott Laboratories at Oak Ridge.

The radioiodinating procedure is described in general terms in the following statement released by the Abbott Laboratories: "The iodinations were carried out by Mr. G. I. Gleason of the Department of Radioactive Pharmaceutica's by means of nascent iodine between 0 and 5° C, in an essentially neutral aqueous medium. Following iodination the reaction is allowed to stand until complete, and then passed over an ion exchange column, capable of quantitatively removing the iodide and the iodate present in the reaction mixture. In general, the amount of these impurities has not exceeded one or at the most two percent, as determined either by dialysis or by filter paper chromatography. For the latter purpose the material is placed upon a narrow strip of Whatman filter paper and is developed by ascending dilution with 70 percent alcohol. The radioactive bands are counted, one centimeter at a time, and the amount of the activity in these bands compared with that of the origin (Tabern, 1953)."

Iodine from 50 micrograms of potassium iodide was incorporated into 100 milligrams of globulin, corresponding to approximately 1 atom of iodine per molecule of protein. This low iodination ratio is well within the stability limits for radio-bound protein. The specific activity of the radioactive material in solution was on the average from 0.1 to 0.25 mc./cc. with an average globulin concentration of 3.5–5.0 mg./cc. Regardless of the concentration of the original serum, the rabbit dosage was based on dry weight of the gamma globulin. A single dose containing approximately 2.0 to 2.5 mg. in 0.5 cc. of saline was injected intravenously into each rabbit.

Preparation of blood and tissue samples. Blood was drawn from the ear vein of rabbits at one hour, and at 1–2 day intervals thereafter until the animals were sacrificed. Duplicate samples of 0.2 cc. of blood were weighed in metal

* I*RGG refers to radioiodinated rabbit gamma globulin.
The radioactivity concentrations in the blood and tissues of the treated rabbits were then measured and evaluated.

bottle caps, digested with 0.1 cc. of N NaOH, and dried overnight at 37° C. For radioactivity of tissues animals in each group were sacrificed at 4, 8 and 14 days after injection with radioactive gamma globulin. For this purpose rabbits were anesthetized with "Nembutal" sodium (25 mg./kg.) injected intra-abdominally with heparin and thoroughly perfused. For perfusion 2 liters of 0.9% saline were passed by gravity through a canula inserted into the abdominal aorta with the perfusate leaving by incision in the *vena cava* until the organs appeared bloodless. Tissues were then removed, thoroughly minced with scalpel and prepared for measurements of radioactivity in duplicate 0.2 gm. samples as described for blood.

Considerable importance was attached to an exhaustive perfusion to ascertain values which were actually attributed to the specific activity of the tissue. It has been previously estimated that the concentrations of radioactivity in the unperfused tissues were greater than in the perfused tissues by an amount equivalent to the blood in the tissue (Pressman, 1949). However the principle of perfusion has not always been observed by other investigators (Latta, 1950; Laws and Wright, 1952).

Preparation of urine samples. For the evaluation of radioactivity excreted in urine immune and non-immune rabbits were kept separately in metabolism cages for the 15 day period. Every 24 hours the total urine output was recorded and 0.2 cc. samples in duplicate were prepared for counting following the procedure described for blood.

Radioactivity measurements. The grade of radiation emitted from radioiodine in blood and tissues was measured by the thin mica window Geiger-Mueller TGC-2 tube with Tracerlab 64 Scaler. Counts per minute per gram of wet tissue were corrected for background and for radioactive decay to time of injection and normalized to 3 kg. rabbit.

Experimental Results

a. *Radioactivity in blood.*

In the first series eight rabbits were injected intravenously with a single dose of 2.41 mg. of anti-spotted fever I*RGG in 0.5 cc. saline (262 microcuries). Of these animals three were non-immune, three were vaccinated against spotted fever and two against epidemic typhus (table 1; fig. 1). A uniformly high radioactivity blood level (about 159,000 cpm./g.) was found at the end of the first hour after injection. This was followed by a precipitous drop during the first 24 hours with the loss of about 50% radioactivity from the blood. This first "dilution phase" (Dixon *et al.*, 1951) which is common to elimination curves for heterologous as well as homologous proteins represents the disappearance rate of anti-spotted fever I*RGG and is followed by a gradual slope of the curves over the 14 day period of radioactivity measurements. No significant differences in the elimination rates were noted

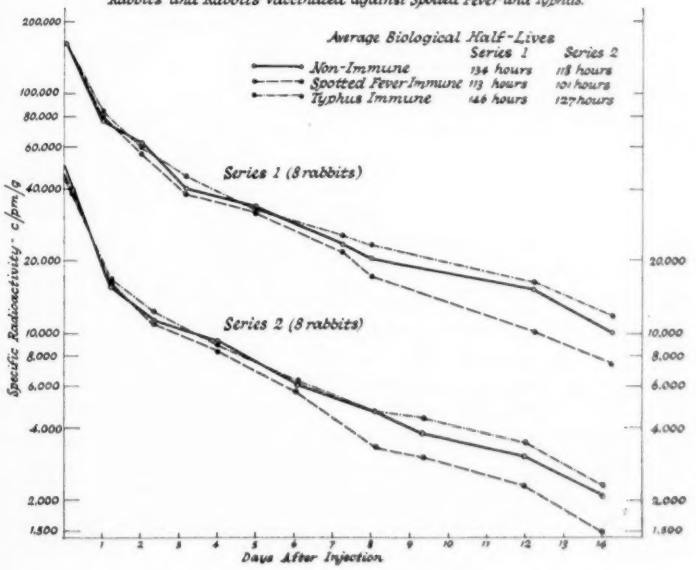
TABLE 1 (Series 1)

Radioactivity concentrations in blood of non-immune and immune rabbits injected with I 131 anti-spotted fever rabbit gamma globulin. Initial dose: 2.41 mg = 262 microcuries = 2.42 x 10^7 cpm.

	Average total counts (cpm/g)		
Time after injection	3 non-immune rabbits	3 spotted fever immune rabbits	2 typhus immune rabbits
1 hour	158,800	159,000	159,800
1 day	76,481	77,615	80,800
2 days	61,321	55,693	58,982
3 days	39,677	38,178	45,229
5 days	32,902	26,820	32,594
7 days	23,244	21,769	25,449
8 days	20,379	17,415	23,080
12 days	15,179	10,348	16,305
14 days	10,263	7,472	11,949

during the first 6 days, but beginning with the 7th day a more rapid decline in radioactivity in the blood of spotted fever immune rabbits was recorded. At this stage the course of the blood level values was diverging from the base line level of the non-

Fig 1. Logarithmic Elimination Curves for Radioiodinated Homologous Anti-Spotted Fever Gamma Globulin in the Blood of Non-immune Rabbits and Rabbits Vaccinated against Spotted Fever and Typhus.



immune animals (half-life 134 hours) indicating an accelerated elimination for I*RGG in spotted fever immune rabbits (half-life 113 hours) and a delay in the clearance for the typhus immune rabbits (half-life 146 hours).

The second series of experiments includes eight rabbits of which two were non-immune, three were immunized against epidemic typhus and three against spotted fever. Each animal was injected with 1.98 mg. of anti-spotted fever I*RGG (232 microcuries) in 0.6 cc. saline (table 2). Counts taken at the end

TABLE 2 (Series 2)

Radioactivity concentrations in blood of non-immune and immune rabbits injected with I 131 anti-spotted fever rabbit gamma globulin. Initial dose: 1.98 mg = 232 microcuries = 191 x 10^7 cpm

	Average total (cpm/g)		
Time after injection	2 non-immune rabbits	3 spotted fever immune rabbits	3 typhus immune rabbits
1 hour	50,462	47,284	44,878
5 hours	39,300	38,824	38,157
1 day, 5 hours	15,700	16,332	16,726
2 days, 8 hours	11,414	10,988	12,437
4 days	9,224	8,528	9,062
6 days, 2 hours	6,128	5,721	6,373
8 days, 5 hours	4,723	3,324	4,709
9 days, 8 hours	3,872	3,068	4,455
12 days	3,085	2,310	3,502
14 days	2,287	1,524	2,349

of the first hour placed this series at a lower initial level of about 50,000 cpm./g., but the trend of the curves shows a striking similarity to the first series (fig. 1). The sharp drop to 40% of the original radioactivity in the blood represents the initial phase in the elimination curves when the equilibration of the I*RGG between vascular and extravascular components of the plasma protein takes place (Dixon *et al.*, 1951). A continuous slope in the course of the curves followed until the sixth day when the curve for spotted fever immune rabbits diverged from the others suggesting an accelerated disappearance rate for I*RGG in the blood. This separation from the base line was still more accentuated when compared with the curve for the typhus immune rabbits. The identical trend of the two series (fig. 1), particularly the synchronic deviation of the spotted fever curves suggests specificity of the elimination rates in the immune rabbits.

In the third series nine rabbits were injected with antityphus I*RGG. Of this group three rabbits were non-immune controls, three were immunized against epidemic typhus and three against spotted fever. The injection of 2.61 mg. antityphus I*RGG in 0.5 cc. (261 microcuries) per rabbit was followed also by a rapid drop in blood radioactivity during the first 24 hours at the end of which approximately 50% of the initial activity (about 184,000 cpm./g.) remained in the blood (table 3). This phase continued

TABLE 3 (Series 3)

Radioactivity concentrations in blood of non-immune and immune rabbits injected with I 131 anti-typhus rabbit gamma globulin. Initial dose: 2.61 mg = 261 microcuries = 2.89 x 10^7 cpm

	Average total counts (cpm/g)		
Time after injection	3 non-immune rabbits	3 typhus immune rabbits	3 spotted fever immune rabbits
1 hour	184,320	178,730	180,120
18 hours	103,440	100,113	102,992
1 day	88,827	82,731	87,579
2 days	68,297	57,045	60,164
4 days	42,844	37,834	38,739
6 days	30,706	27,632	28,628
8 days	20,445	17,939	23,231
11 days	16,360	14,926	18,772
13 days	14,500	12,193	16,815
14 days	12,243	9,113	15,225

a parallel course until the 6th day, when a deviation of the curve for typhus immune rabbits (half-life 139 hours) from the curves for the non-immune rabbits (half-life 146 hours) and for spotted fever immune rabbits (half-life 163 hours) was recorded.

The more rapid loss in radioactivity in the specific immune rabbits becomes also evident when the average total counts in the blood represented by the middle columns of tables 1, 2 and 3 are compared with the counts of the non-immune and unspecific immune animals. Marked differences were found on the 14th day after injection in all 3 series with counts reduced by 28%, 33% and 27% respectively in immune rabbits as compared with the controls (tables 1, 2, 3).

The fourth series of fifteen rabbits was divided in three groups, of which five rabbits were immunized against spotted fever, five against epidemic and murine typhus with a mixed vaccine and five were untreated serving as controls (table 4, fig. 2). Each rab-

TABLE 4 (Series 4)

Radioactivity concentrations in blood of non-immune and immune rabbits injected with I 131 anti-spotted fever rabbit gamma globulin. Initial dose: 144 mg = 190 microcuries = 2.19 x 10^7 cpm

Time after injection	Average total (cpm/g)		
	5 non-immune rabbits	5 spotted fever immune rabbits	5 typhus immune rabbits
1 hour	79,892	81,086	81,968
1 day	37,017	37,143	39,291
2 days, 2 hours	26,936	23,209	29,637
3 days	22,805	19,299	21,581
4 days, 1 hour	19,050	17,506	18,638
5 days, 7 hours	16,979	15,012	16,274
6 days, 4 hours	16,340	12,100	14,989
7 days, 2 hours	12,700	9,380	10,528
8 days, 8 hours	11,125	7,832	8,424
9 days, 11 hours	9,583	6,237	7,421
11 days, 9 hours	8,184	5,092	6,356
12 days, 8 hours	7,769	4,426	5,804
14 days, 2 hours	5,852	2,914	3,390
15 days, 9 hours	4,998	2,236	2,879

bit of the entire series was injected with 1.44 mg. anti-spotted fever rabbit gamma globulin tagged with 190 microcuries I 131. There was the usual drop in radioactivity at the end of the first 24 hours corresponding to the first phase of the previous series of ex-

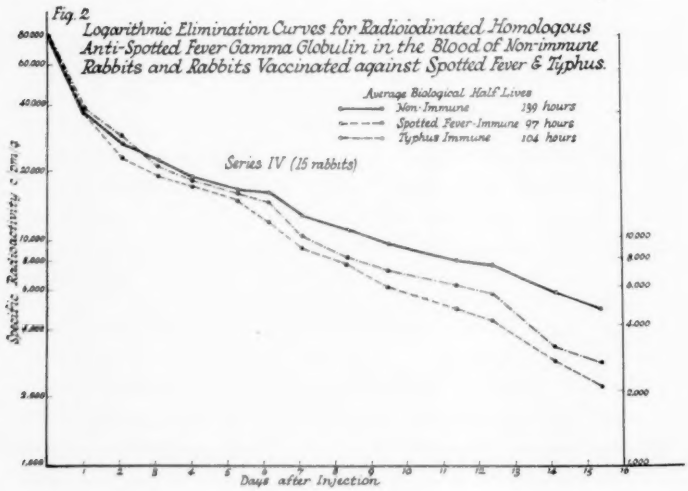


Fig. 2
Logarithmic Elimination Curves for Radioiodinated Homologous Anti-Spotted Fever Gamma Globulin in the Blood of Non-immune Rabbits and Rabbits Vaccinated against Spotted Fever & Typhus.
Average Biological Half Lives
Non-Immune 139 hours
Spotted Fever-Immune 97 hours
Typhus Immune 104 hours
Series IV (15 rabbits)
Specific Radioactivity c/m/g
Days after Injection

periments. However, at this point the deviation of the elimination curves for the immune rabbits became more evident indicating an accelerated rate of elimination as compared with the base line of the controls. This applies to the spotted fever curves as well as to typhus immune rabbits. Similarly to the curves for the first two series (fig. 1), a more accentuated separation in the immune rabbits is noted about the sixth day after injection, suggesting perhaps the "third phase" of the elimination curves. This section which is clear-cut and characteristic for the clearance curves of heterologous proteins, ordinarily does not develop in the case of homologous proteins. The accelerated disposal of radioactivity continued during the entire period of observation over 15 days of daily evaluation of blood samples. Thus a half-life of 97 hours for spotted fever immune rabbits, which is 30% less than the half-life of gamma globulin injected into non-immune rabbits, was established. Similarly, the half-life for typhus immune rabbits, was in this series shortened to 104 hours (25% less) against 139 hours of the controls (fig. 2). A reduction by 50% of total counts in spotted fever immune rabbits as compared with non-immune controls was noted on the 15th day after injection with I 131 gamma globulin (table 4). In this regard the elimination rates for typhus immune rabbits of series 1 and 2 (fig. 1) differ from the corresponding curves of series 4, the former showing a delayed disappearance rate and longer half-life as compared with the curve for normal rabbits. This apparent discrepancy with series 4 may be due to the fact that the typhus immune rabbits of series 4 were immunized with a mixed typhus vaccine (epidemic and murine type) with a total of 12 injections given intravenously, subcutaneously and intramuscularly over a period of 3 months as compared with the usual 5 injections given in three weeks. Since the degree of active immunity determines the characteristics of the elimination curve (Dixon *et al.*, 1951), it is feasible to assume that the clearance rate for anti-typhus gamma globulin was influenced in series 4 by the more complete immune responses of the hyperimmune typhus rabbits.

Parallel experiments were conducted with normal I*RGG which was injected into non-immune and typhus immune rabbits. Radioactivity blood determinations indicated that only minor differences existed in the biological half-lives in the two groups within the range of 118 to 122 hours. Furthermore, radioactivity

values for the various tissues failed to show any marked differences in the elimination rates for the injected normal I*RGG in non-immune and immune rabbits.

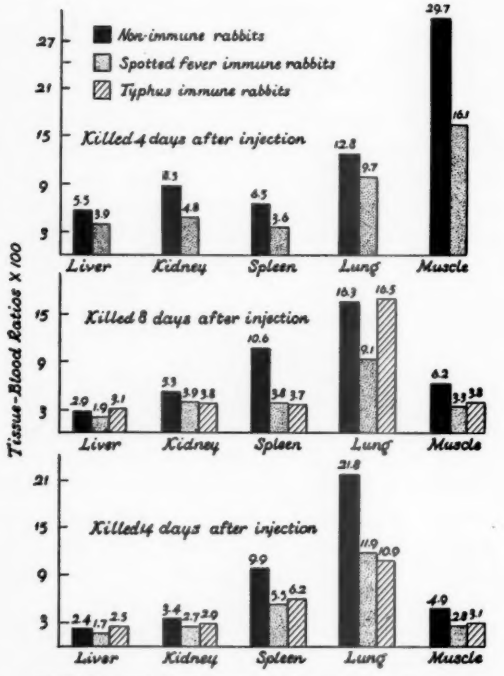


Fig. 3. Radioactivity Concentrations Expressed in Tissue-Blood Ratios (T/B) for Tagged Anti-Spotted Fever Rabbit Gamma Globulins in Various Tissues of Non-immune Rabbits and in Rabbits Vaccinated Against Spotted Fever and Typhus.

In view of the variable elimination rates for antityphus I*RGG in the blood most probably due to the various degree of immunity of the rabbits, the limited statistical data for the typhus series are not significant. However, the consistency of the results showing the constant accelerated elimination rates for spotted fever I*RGG in the blood of specifically immunized rabbits is demonstrated by the statistical analysis. The mean half-life of 102.4 hours for spotted fever I*RGG in the blood of eleven rabbits immunized against spotted fever as compared with the mean of

131.4 hours in ten normal controls is expressed by "t" value 4.07 with probability (P) less than 0.001.

b. *Radioactivity in tissues.*

The relative radioactivity concentrations in tissues (liver, kidney, spleen and lung) were evaluated by the tissue/blood ratio (T/B) obtained by dividing the counts for various tissues by the values for the blood of the same rabbit (Eisen and Pressman, 1950). This index proved a valuable criterion for comparative evaluation of the degree of retention of radioactivity by the tissues.

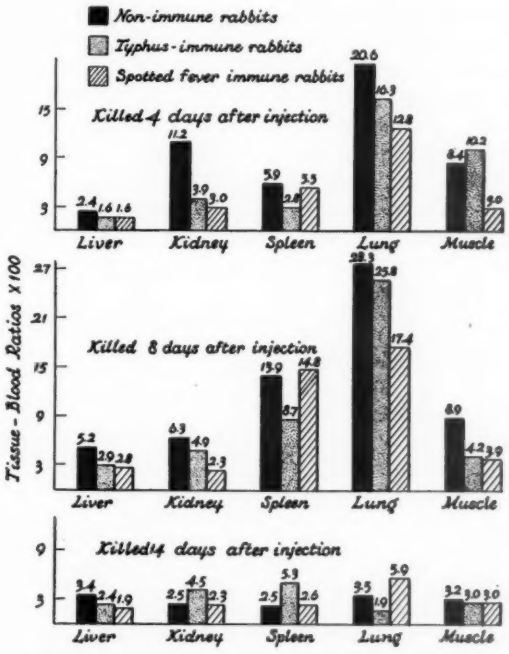


Fig. 4. Radioactivity Concentrations Expressed in Tissue-Blood Ratios (T/B) for Tagged Anti-typhus Fever Gamma Globulins in Various Tissues of Non-immune Rabbits and in Rabbits Vaccinated against Typhus and Spotted Fever.

The T/B ratio is especially significant during the first several days after injection. It serves as a value which corrects for varia-

tions in the amounts injected and also for variations in body weight (Eisen, Sherman and Pressman, 1950). In the majority of tests the ratios indicated relatively lower levels of radioactivity in immune rabbits as compared with the non-immune animals (fig. 3, 4). In this regard the liver of the spotted fever immune rabbits injected with anti-spotted fever I*RGG showed the lowest values as compared with other tissues (fig. 3). Similar low values were noted for the kidney on the 4th and 14th day after injection.

Calculations of radioactivity in typhus immune rabbits on the 4th, 8th and 14th day after injection of typhus I*RGG showed in the majority of instances lower values than in non-immune rabbit (fig. 4). This was corroborated by a series of experiments in which I*RGG against epidemic and murine typhus was used. Differences in radioactivity for the various tissues(liver,spleen, lung, kidney, muscle) of non-immune and typhus immune rabbits were significant, indicating constantly lower levels of remaining radioactivity in immune rabbits as compared with controls (tables 5 and 6). For example, the radioactivity of the liver

TABLE 5

Radioactivity concentrations in tissues of non-immune and immune rabbits injected with I 131 anti-spotted fever rabbit gamma globulin

Rabbit No.	Immunity	Days after injection	Radioactivity in tissues (cpm/g)					
			Blood	Liver	Kidney	Spleen	Lung	Muscle
62	none	4	32,757	1,846	2,783	2,105	4,189	9,720
56	Spotted fever	..	33,447	1,333	1,591	1,190	3,242	5,380
63	none	8	20,379	602	1,092	2,169	3,331	1,254
57	Spotted fever	..	17,415	388	676	662	1,591	580
51	Typhus	..	23,080	705	888	865	3,814	879
64	none	14	10,263	245	345	1,015	2,229	507
58	Spotted fever	..	7,472	126	198	410	890	287
52	Typhus	..	11,949	302	349	743	1,312	371

of epidemic typhus immune rabbit was 35% that of the normal rabbit on the 7th day after injection with typhus gamma globulin. At the same time the radioactivity in the kidney and spleen of the immune rabbits was only 26% and 31% respectively that of corresponding tissues of the non-immune rabbits.

Similar observations based on T/B ratios were made on rabbits immunized against murine typhus and subsequently injected with tagged murine typhus RGG. The concentrations of radioactivity in liver, kidney and spleen were on the 8th day after injection about 50% lower than in tissues of non-immune rabbits. This general phenomenon of lower values in immune rabbits as shown by the remaining tissue radioactivity is interpreted as the result of higher elimination rate for the gamma globulin and depends, according to Dixon *et al.* (1952), on the metabolic rate of the host. Our observations on the fate of homologous gamma globulin in non-immune and sensitized rabbits agree with those of Laws and Wright (1952) on the disposal of radioiodinated heterologous proteins (bovine gamma globulin), particularly in liver and spleen. Counts obtained by these authors in liver, spleen, kidney and muscle from specifically sensitized animals after injection with tagged proteins were similar to our results, much lower than in controls. The total counts for radioactivity in the tissues showed considerable differences between the organs of the same rabbit and measured at the same time (figs. 3 and 4). The concentrations in the tissues were at different intervals 1/10 to 1/30 of the corresponding blood values.

TABLE 6

Radioactivity concentrations in tissues of non-immune and immune rabbits injected with I 131 anti-typhus rabbit gamma globulin

Rabbit No.	Immunity	Days after injection	Radioactivity in tissues (cpm/g)					
			Blood	Liver	Kidney	Spleen	Lung	Muscle
67	none	4	50,785	1,205	5,669	2,977	10,470	4,237
54	Typhus	..	44,605	730	1,765	1,260	7,277	4,540
59	Spotted fever	..	49,409	772	1,496	2,722	6,316	1,490
65	none	8	19,095	993	1,200	2,647	5,401	1,699
53	Typhus	..	17,939	530	873	1,555	4,618	758
60	Spotted fever	..	21,956	627	496	3,256	3,807	861
66	none	14	12,243	421	301	304	426	396
55	Typhus	..	9,113	218	406	485	175	269
61	Spotted fever	..	15,225	288	344	397	900	459

Similar observations were made by Latta (1950) who found high concentrations of radioactivity in the lung and kidney of rabbit injected with foreign proteins. However Latta attributes almost all the activity to blood in vascular tissues like lung.

Since contrary to Latta's technique, in our experiments blood has been eliminated by exhaustive perfusion, our values for radioactivity can be attributed entirely to specific tissue activity. The highest concentrations of radioactivity were found in our series in the lung and muscle of normal rabbits on the fourth day after injection when blood levels were 5–10 times higher. The next in order of radioactivity were the kidney, spleen, and liver, the latter showing an average of 1/15 of blood radioactivity on the fourth day. With the decrease of the tissue values with time, the highest concentration was retained by the lung and spleen as late as the 14th day. At the same time the radioactivity concentrations of most of the immune rabbits were with two exceptions considerably lower. These results indicate that the tissues of rabbits sensitized with specific rickettsial antigen show a lower retention rate for the corresponding antibody represented by the homologous gamma globulin.

c. *Radioactivity in urine.*

For preliminary experiments urine from 2 rabbits of the spotted fever immune series and from 2 non-immune rabbits was col-

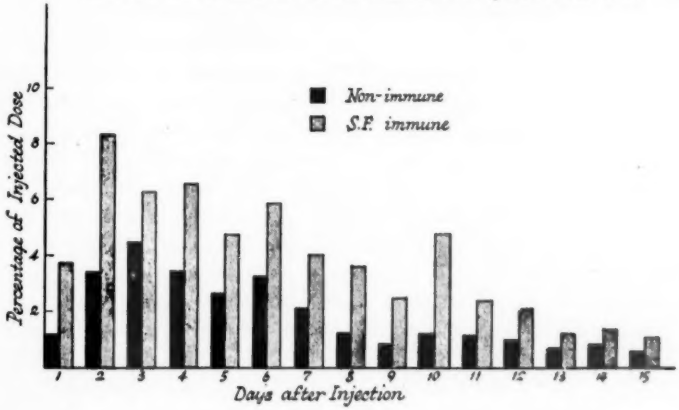
TABLE 7

Radioactivity excreted in urine of non-immune and immune rabbits injected with I 131 anti-spotted fever rabbit gamma globulin. Initial dose: 1.44 mg = 190 microcuries = 2.19 x 10^7 cpm. Average values at 24 hour periods after injection.

	Non-immune rabbits (one pair average)		Spotted fever immune rabbits (one pair average)	
24 hr. period after injection	Total counts	% of the injected dose	Total counts	% of the injected dose
1st	2.53 x 10^5	1.15	8.18 x 10^5	3.74
2nd	7.44	3.39	18.21	8.31
3rd	9.81	4 46	13.74	6.27
4th	7.58	3.46	14.40	6.57
5th	5.90	2.69	10.38	4.75
6th	7.20	3.28	12.90	5.89
7th	4.78	2.18	8.96	4.09
8th	2.78	1.27	7.95	3.63
9th	1.88	0.85	5.50	2.51
10th	2.74	1.25	10.65	4.84
11th	2.58	1.17	5.32	2.42
12th	2.19	1.00	4.68	2.14
13th	1.59	0.73	2.80	1.28
14th	1.91	0.87	3.15	1.44
15th	1.34	0.61	2.41	1.10
		Total 28.36%		Total 58.98%

lected every 24 hours over a period of 15 days. The radioactivity values of the total of 60 urine samples in duplicate were corrected to total counts for 24 hours excretion and expressed in percentage of the initially injected dose of I*RGG. Urine samples at 24, 48 and 72 hours were prepared in 3 different ways: intact urine as excreted, urine after centrifugation for 20 minutes and finally, after precipitation of protein with an equal volume of 30% trichloracetic acid (Laws, 1952). Irrespective of the method used inclusive of the test for protein-bound radioactivity there were no significant differences in radioactivity values. Consequently, since the radioactive iodine excreted in urine appears to be non-protein-bound, samples after 72 hours were prepared from intact urine only. The total daily counts of radioactivity in urine of non-immune rabbits indicate, that the peak (9.81×10^5 cpm) is reached at the end of 72 hours after injection (table 7) as compared with the peak (18.21×10^5 cpm) reached in the spotted fever immune rabbits at the end of 48 hours (fig. 5). This level was followed by a steady decline in radioactivity, but as a consistently higher level in the immune rabbits as compared with the non-immune animals. The excretion rate of radioactivity amounts to 28.36% of injected dose in the non-immune rabbits as compared with 58.98% in spotted fever immune rabbits.

Fig. 5 Excretion of Radioactivity in Urine of Non-immune Rabbits and in Rabbits Immunized against Spotted Fever (S.F.)



These results are comparable in principle with observations of Laws and Wright (1952) on excretion in urine of inorganic radioactive iodine originally bound to bovine serum albumin; however, urinary excretion of radioactive iodine after protein breakdown proceeded much more rapidly than in our experiments. In other words, the more rapid urinary excretion of radioactivity is characteristic for sensitized rabbits whether the tracer was originally bound to heterologous protein (antigen) or to homologous gamma globulin (antibody). In either case the rate of excretion depends on the immune catabolism of the respective protein and at least in part on the metabolic rate of the host.

Summary and discussion

The distribution and fate of radioiodinated antirickettsial homologous serum gamma globulin were investigated in the blood, tissues and urine of non-immune and specifically immunized rabbits. The study involves a total of 46 rabbits of which 16 were non-immune controls, 14 were immunized with a specific vaccine against spotted fever and 16 were vaccinated against typhus. All rabbits were injected with a single dose (2.0–2.5 mg.) of I 131 antirickettsial homologous gamma globulin (I*RGG). In addition, normal rabbit gamma globulin was used in control experiments. The elimination rate for the injected I*RGG was determined from logarithmic curves based on daily radioactivity measurements of blood samples over a period of 14 days. A sharp drop in radioactivity of the blood within the first 24 hours was a constant phenomenon in all series of the injected rabbits. This phase was followed by a sloping curve representing the disappearance rate for the I*RGG. This curve diverted in immunized rabbits from the base line level of normal rabbits at the end of the first week indicating an accelerated elimination of I*RGG from rabbits immunized against spotted fever or typhus. Radioactivity counts were constantly lower in the blood of immune rabbits (from 27% to 56%) as compared with non-immune animals injected with the same I*RGG. Radioactivity concentrations in tissues of perfused animals were evaluated by the tissue/blood ratio in dividing the counts for various tissues by the values for the blood of the same rabbit. Reduced levels of radioactivity indicating higher elimination rate for I*RGG were also characteristic for tissues of rabbits immunized against spotted fever, epidemic

or murine typhus. Lowest values were obtained for the liver of immune rabbits (35% to 50% that of controls). No such differences were found in blood or tissues of normal or immune rabbits injected with normal I*RGG.

The percentage of the injected dose of radioactivity excreted in urine in non-protein-bound form showed considerable differences in non-immune and immunized rabbits. Whereas 28.36% of the injected dose was excreted in non-immune rabbits, the spotted fever immune rabbits excreted as much as 58.98% radioactivity of the injected dose. The more rapid disappearance of antirickettsial homologous gamma globulin from the system of actively immunized rabbits follows a similar although more vigorous clearance in the elimination of foreign proteins. This clearance is apparently due to the immune response of the host and to the appearance of the antibodies which after combining with the antigen are rapidly eliminated (Talmage *et al.*, 1951).

In the present study the elimination of the passively administered homologous gamma globulin (antibody) in non-immune rabbits followed the two-phase course observed by previous investigators. However, a distinct acceleration in the elimination rate (figs. 1 and 2) suggests a "third phase" in the curve for the immune rabbits. The interpretation of this phenomenon gives rise to speculation as to the possible persistence of the residual rickettsial antigen, including the immunizing soluble 'S' component of the rickettsial vaccine. Thus the tissues of sensitized rabbits contribute to the immune catabolism and to the accelerated elimination of the gamma globulin.

Acknowledgments. The authors greatly appreciate the advice and assistance rendered by Dr. J. Allen Scott and Miss Mary Westbrook in the statistical analysis of this study. Thanks are due to Dr. W. W. Nowinski, Director, Tissue Metabolism Laboratory, for the electrophoretic analysis of the sera. The authors are indebted to the Lederle Laboratories Division for the generous supply of rickettsial vaccines and immune sera obtained through the courtesy of Drs. Herald R. Cox and J. M. Ruegsegger.

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INFRARED ABSORPTION STUDIES OF VIRUS PREPARATIONS*

ALBERT A. BENEDICT, MORRIS POLLARD AND FRANK B. ENGLEY, JR.

Although the infrared spectra of viruses studies previously (Pollard, *et al.*, 1952) appeared to be different, there was an apparent absence of the sharp absorption bands associated with proteins and complex biological substances (Darmon and Sutherland, 1947; Blout and Mellors, 1949; Randall, *et al.*, 1951; Schwarz, *et al.*, 1952; Stevenson and Bolduan, 1952).

The data contained in the present report indicated that more highly concentrated material was required in order to obtain spectra resembling those reported on other complex biological systems. A more definitive study was made to obtain fractions of infected chorioallantoic fluid which could best serve as a means of identification of viruses, particularly from the standpoint of developing the most expedient method and at the same time revealing reproducible absorption spectra.

Methods. The following viruses were investigated: influenza A (PR–8 strain),[1] influenza A' (FM–1 strain),[1] influenza B (Lee strain),[1] mumps (Enders strain),[1] meningopneumonitis (MP),[1] Newcastle disease (Cal strain),[2] psittacosis (Texas turkey strain).[3] The chorioallantoic cavities of 10-day-old chick embryos were inoculated with 0.05 ml of 10^{-2} — 10^{-6} dilutions of the influenza strains and NDV, and the chorioallantoic cavities of 7-day-old embryos were used for the propagation of mumps (10^{-2}), psittacosis (10^{-2}) and MP viruses (10^{-2}). The inoculated embryos were incubated at 35° C., and all of the embryos, when 12 days old, were refrigerated overnight and the chorioallantoic fluids (CAF) harvested. The fresh fluids were cleared by centrifugation at 3,000 rpm for 30 minutes. The supernates were retained and are referred to as crude CAF.

1 cycle differential centrifugation (1 UC). Crude CAF was centrifuged at 100,000 × g for 30 minutes at 4° C. and the resulting pellets suspended in

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[1] Obtained from the Viral and Rickettsial Registry of the American Type Culture Collection.

[2] Obtained from Dr. Raymond Bankowski, School of Veterinary Medicine, University of California, Davis, California.

[3] Obtained from Dr. J. V. Irons, State Health Department, Austin, Texas.

distilled water at pH 7.0 at either 1/10 or 1/100 of the starting volume of CAF. After standing at approximately 25° C. for 2 hours the suspension was cleared by centrifugation at 3,000 rpm for 30 minutes.

3 cycle differential centrifugation (3 UC). Crude CAF was centrifugated at 100,000 × g for 30 minutes at 4° C. and the pellets were suspended in 0.1 M phosphate buffer at pH 7.0. The use of phosphate buffer at this step instead of distilled water (pH 7.0) gave a better recovery of the viruses, excepting in the case of mumps. The suspension was cleared by centrifugation at 3,000 rpm for 30 minutes and the recovered supernate centrifuged at 100,000 × g for 30 minutes. The pellets were suspended in distilled water and a third cycle of differential centrifugation was applied; the final material being suspended in distilled water at about 1/200 of the starting volume of CAF. The same methods of preparation were used to process normal CAF obtained from 12-day-old embryos. Approximately 250–300 ml of infected CAF and 500–600 ml of normal CAF were required to obtain sufficient material to analyze. At least 4 different batches of each virus were prepared for the infrared analysis.

The infrared absorption spectra from 2–16 μ were determined with a Baird-double beam recording instrument equipped with a sodium chloride prism. The standard liquid micro-cell was adapted to hold a 1 square inch silver chloride plate. Depending upon the material to be analyzed, either 0.2 or 0.3 ml of the preparations were spread evenly over the surface of the silver chloride plate in the shape of the aperture of the micro-cell. The specimens were dried slowly at 36° C. The quality of the films were judged by the use of the maximum transmission value at 5.5 and the minimum value at 6.1 μ (Levine, *et al.*, 1953). For control purposes, infrared analysis of bacteria was performed according to the technique described by Stevenson and Bolduan (1952).

Hemagglutination titers were determined either by the 50% endpoint method of Hirst and Pickels (1942), using a Klett photoelectric colorimeter, or by the 100% endpoint method (Hirst, 1942). One and a half percent and 0.5% chicken red blood cell suspensions were used in the former and the latter techniques, respectively. Concentration of elementary bodies of psittacosis and MP was determined by microscopic examination. Total nitrogen was determined by a modification of the microkjeldahl technique (Kabat and Mayer 1948), in which a Markham type kjeldahl distillation apparatus was used.

Estimation of purity of the virus preparations was not determined since the criterion used for identification of the viral types was based on reproducibility of significant differences. In the case of the influenza strains, however, the efficiency of concentration in terms of hemagglutinin units/mgm. of nitrogen was determined and found to be of the same magnitude as reported by others (Stanley, 1945; Cox, *et al.*, 1947).

Results

The reproducibility of the infrared method for identification of complex biochemical systems was tested by recording the

spectra of several bacteria which had been reported by Stevenson and Bolduan (1952) and by Levine, *et al.* (1953). *Escherichia coli, Aerobacter aerogenes, Bacillus subtilis* and *Sarcina lutea* were thus analyzed. Although variations existed among various preparations of the same bacterial species, the characteristic bands differentiating these organisms were observed. In order to obtain satisfactory films, it was necessary to use about twice the quantity of bacteria as the amount that had been reported (Stevenson and Bolduan, 1952).

Crude CAF. Approximately 0.3 ml. of crude CAF was found to give satisfactory spectra. When the crude fluids from single eggs infected with the same virus and the fluids from single normal eggs were compared, the magnitude of the variation was so great that assigning identifying characteristics, as has been done with bacteria (Stevenson and Bolduan, 1952), was impossible. The variation was evident between 8.5–9.8 and 11.0–13.0μ. In the case of fluids infected with the influenza strains, NDV and mumps the variations had no apparent correlation with viral content, as measured by the 50% hemagglutinin method. Reproducible curves were obtained from those fluids which had, by microscopic examination, showed the greatest concentrations of either psittacosis or MP.

Figure 1 presents the spectra from 6–13μ of pooled infected and normal CAF. The deep bands from 8.6–10.6μ were sufficiently consistent among different pools to at least indicate virus infection when these spectra were compared to the spectrum of normal CAF. NDV was particularly characteristic as shown by the more gradual slope of the absorption curve from 8.0–8.5μ. It was not possible however to differentiate influenza A and mumps from normal CAF, nor MP from psittacosis.

It was noteworthy that the supernates of the pooled CAF, obtained by centrifugation of the CAF at 100,000 $\times$ g for 45 minutes, yielded the same spectra as did the uncentrifuged fluid. Centrifugations at this speed removed most of the infective particles and all of the hemagglutinin, and thus, it would be reasonable to conclude that the variations observed were reflections of host metabolic products and/or viral products (soluble antigens), rather than actual physico-chemical properties related to the virus particles.

It had been difficult to reproduce accurately the quantity of absorbing material in a series of pooled fluids. The variation in the amount of light scattering was probably due to the varying concentrations of the absorbable substances. Subsequently more concentrated preparations did not exhibit as great a variation in either the amount of energy being transmitted or the amount of light being scattered.

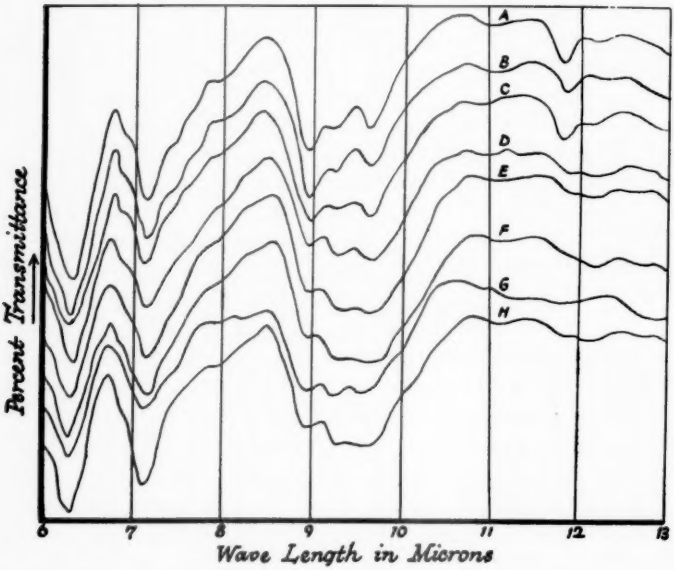

FIG. 1. Spectra of virus-infected and normal crude CAF: A, psittacosis; B, meningopneumonitis; C, influenza A'; D, influenza B; E, influenza A; F, mumps; G, Newcastle disease; H, normal.

1 UC preparation. The experimental procedure of Pollard, *et al.* (1952) consisting of a single high speed centrifugation of the cleared CAF, and suspension of the pellets in water to 1/10 of the original CAF volume was repeated. The spectra produced by these preparations reflected the use of materials of insufficient concentration to produce distinct absorption bands. This fact was particularly evident with the smaller viruses. When, however, these fractions were suspended in distilled water to 1/100

of the original CAF volume (60 ml), spectra comprised of sharp peaks and "shoulders" were obtained. Figure 2 presents the spectra of normal CAF and influenza A prepared by this method.

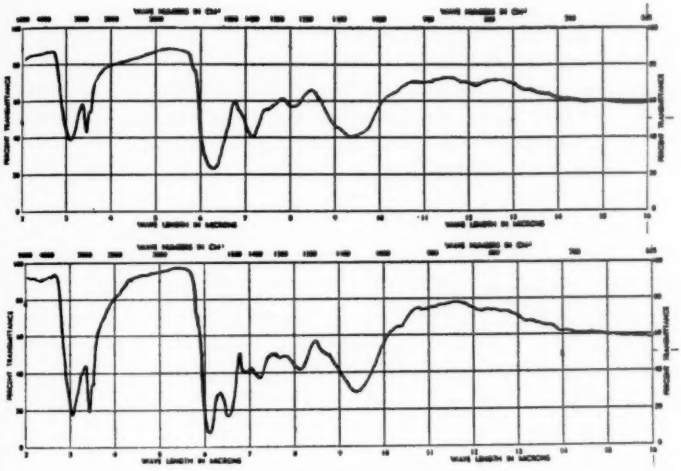

FIG. 2. Spectra of normal CAF (top) and influenza A (bottom) prepared by 1 cycle of differential centrifugation.

Similar to the situation with crude CAF, reproducibility of the spectra obtained by this method was poor. It was possible however to differentiate infected material from normal host components. With preparations of influenza A having high hemagglutinin titers the absorption bands at 6.5, 6.9, 8.1 and 8.7μ were resolved, and the broad band at about 9.3μ showed a greater absorption (fig. 2). Since certain features of the spectrum appeared to relate to virus content, infrared anaylsis may prove to be a valuable tool for following purification of viruses.

3 UC preparation. The spectra of different samples of the viruses prepared by 3 cycles of differential centrifugation were found to be qualitatively reproducible. It was possible therefore to detect subtle differences that existed among some of the different virus preparations studied. Figure 3 shows the most informative region of the spectra (6–12μ) of viruses and normal CAF. Although we can assume that virus concentration and partial

purification had been effected, as was indicated by decrease in total nitrogen and increase in hemagglutinin, consistent differences among the influenza strains and mumps virus preparations were not detected. Newcastle disease virus showed similar major absorption peaks; however, it was possible to distinguish this virus consistently by the shape of the curve in the region of 8.8μ. Meningopneumonitis was easily identified by its characteristic spectrum (fig. 3). Spectrum of normal CAF appeared to be the same as the spectra of the influenza strains and mumps virus. Ap-

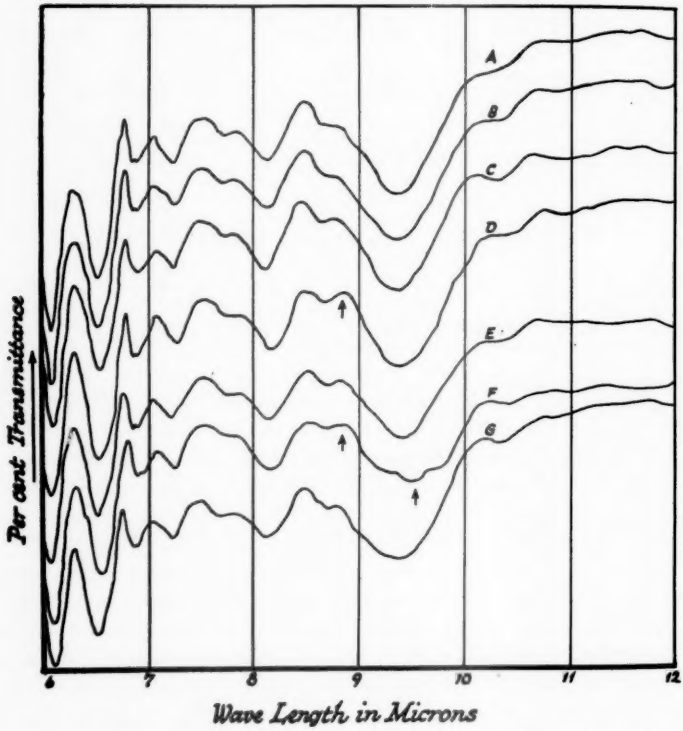

FIG. 3. Spectra of virus-infected and normal CAF prepared by 3 cycles of differential centrifugation: A, influenza B; B, influenza A; influenza A'; D, Newcastle disease; E, mumps; F, meningopneumonitis; G, normal. Arrows refer to distinguishing features of Newcastle disease and meningopneumonitis preparations.

parently the purification procedure employed had reduced the influenza, mumps and normal host preparations spectrally similar components. It was felt that of all the fractions studied to date, the 3 UC preparations were the most promising for the identification of viruses.

Discussion. The use of crude fractions for the identification of viruses by infrared absorption spectroscopy is complicated by the inherent varying ratios of host material to virus content. For example, the marked variation of the spectra of crude CAF observed among individual normal embryos suggested that the host products existed in varying concentrations and that the viruses were not being propagated in a spectrally homogeneous medium. Although the incubation time may be the same for a group of eggs, a lag in development can occur among some of the embryos. Consequently, the concentration of metabolic products in the CAF will vary according to the age of the embryo, and thus, may explain the poor reproducibility of normal CAF. Standard conditions, such as, control of temperature and culture medium, are of utmost importance for infrared reproducibility (Stevenson and Bolduan, 1952).

Similar to the crude normal CAF, the spectra of different individual infected eggs of the same virus were not the same, although some "ironing out" of these inconsistencies resulted from pooling of normal or of infected fluids. The variation noted with the 1 UC preparations appeared to be related to the hemagglutinin activity, whereas, the variation of the crude CAF was not correlated with either the hemagglutinin titers or the sedimented components. Regardless of the constituents that were being measured, a control of the virus content in crude fractions must be made, since the subtle differences that may possibly exist among different viruses would be influenced by the activity of the viruses in the chick embryo.

The reproducibility of the 3 UC fractions was sufficiently consistent to allow the identification of NDV and MP among the viruses studied. This purification procedure however had reduced the influenza and mumps preparations to spectrally common components. These components may well represent host material, as was evidenced by the almost identical spectrum of the complex obtained from normal CAF. The data were compatible with the findings that purified influenza preparations contained a large

proportion of normal host materials (Knight, 1944; Cohen, 1944); the host components existing either as contaminants or as integral parts of the virus particles. Futhermore, the infrared analysis confirmed the results of other physical analyses (Stanley and Lauffer, 1952) relative to the intimate relationship of the host and viral constitutents. It would follow then, since with the 3 UC preparations the normal host preparations and influenza and mumps virus preparations showed the same infrared spectra, that the variations in the host/virus ratios would not be detected as had been noted with less pure fractions.

Not all viral preparations are as closely related to the normal host constiuents, as it was observed that the 3 UC preparations of NDV and MP were distinct from the normal host fraction. These viruses kill the embryos and possibly host products resulting from the death of the embryos were being detected. Contrary to this idea, the same spectrum was secured with the 3 UC fraction of MP prepared from living embryos as was obtained from a similar fraction of MP which had been harvested from dead embryos.

It has been pointed out by Levine, *et al.* (1953), among others, that the interpretation of infrared spectra of complex mixtures is difficult. Similarly, the interpretation of our analysis is complicated by the heterogeneous nature of the complexes that were studied. The infrared spectra of different proteins are similar (Sutherland, 1952). Consequently, the dominance of the proteins can mask the substances of smaller concentration. It was apparent however that the distinct spectrum of MP was due to the relatively larger size of this virus, and thus, the concentration of sufficient virus material has masked the absorption due to host substances. Although the particle size of NDV is similar to that of the influenza and mumps viruses, the more prolific growth of NDV made possible the concentration of adequate viral material to influence the spectrum. It is possible that the purified viruses of the LGV-psittacosis group will yield characteristic spectra, as evidenced with that of MP. These studies are in progress and will be the subject of a subsequent report.

Summary. The infrared absorption spectra of crude CAF infected with influenza strains, mumps, NDV, psittacosis and meningopneumonitis viruses were not sufficiently reproducible for the identification of these viral types. Except for influenza A and

mumps the spectra of these viruses were different than the spectrum of normal CAF. Spectra of virus fractions prepared by 1 cycle of differential centrifugation were not sufficiently reproducible to allow identification of the viruses. The infrared spectra were identical for normal CAF, influenza A, influenza A', influenza B and mumps viruses which had been processed by 3 cycles of differential centrifugation. Newcastle disease and meningopneumonitis viruses, similarly prepared, showed characteristic absorption spectra. The significance of these findings are discussed.

It is a pleasure to acknowledge the technical assistance of Mr. James Jackson.

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A PSYCHOLOGY PROGRAM IN A CANCER RESEARCH HOSPITAL*

BEATRIX COBB, R. LEE CLARK, JR., C. D. HOWE, AND J. B. TRUNNELL

A psychology program in a cancer research hospital is a departure from the usual psychological endeavors in a mental institution. The organization of such a program highlights a new area of service and research for psychologists. It also affords a medical training facility to supplement and enhance regulation requirements for the doctoral degree for those interested in medical-psychological fields while gaining experience in supervised research and service to chronically ill patients.

The program at M. D. Anderson Hospital for Cancer Research has been designed and developed toward such a two-fold goal. First, it has been constructed to fit the needs of a particular institution—a hospital dedicated to the care and understanding of a specific disease, cancer. Second, it has been designed to train psychologists working in such a setting in basic factors in medicine and cancer essential to adequate research or service skills in the area.

In order to describe such a program effectively, it is necessary to understand the historical background of the movement. Following a resume of the history of the idea toward the incorporation of a psychology program into a research hospital setting devoted exclusively to cancer, the three-dimensional program as it now operates will be described. Finally, a projection of the final goal of a well-rounded, interdisciplinary social science research and service program will be presented.

History of the Movement

Cognizant of recent trends in the fields of medicine and psychology toward a psychosomatic orientation, it was the Director of M. D. Anderson Hospital who took the initiative in establishing a psychology program as an integral part of a cancer research hospital. In 1948, he first called a cross-disciplinary

* From the M. D. Anderson Hospital for Cancer Research, The University of Texas, Houston. Received for publication December 10, 1953.

meeting, to which representatives from sociology, psychology, psychiatry, and medicine were invited.[1] The purpose of the conference was to discuss the possibilities of research and service in the area of psychology in the medical framework of the M. D. Anderson Hospital program. From this meeting, a tentative project was activated.

In February of 1951, a psychologist became a member of the research medical staff, being appointed to the hospital under a graduate fellowship from The University of Texas. The psychologist was assigned to the Department of Medicine as a member of that department. In addition to this institutional supervision, the psychologist worked under the guidance of an interdisciplinary advisory council composed of a physician, a psychiatrist, a sociologist, and a psychologist.[2]

The psychologist's assignment was a dual one. First, exploratory research in cancer patient behavior was the major task. Second, an investigation of possible areas of service and research in which a psychologist might assist toward a more efficient offensive against cancer was to be reported.

The preliminary investigation was reported at the end of seventeen months of intensive research. It was realized that medical-psychological research in cancer is only in the beginning stage. Further inquiries are necessary to separate out psychological attributes which emerge along with the course of the neoplasm and those possibly present in a pre-cancer period. Inter-disciplinary theories, hypotheses, and research operations must be developed to clarify relationships among organic and psychological states, as well as the influences of social background and cultural experiences upon cancer patients. These

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[1] Members of the initial group called together by Doctor R. Lee Clark, Jr., M.D., Director of M. D. Anderson Hospital and Surgeon in Chief, were as follows: Robert L. Sutherland, Ph.D., Director, The Hogg Foundation for Mental Hygiene and professor of sociology; Lamar Ross, M.D. (general practitioner); Ernestine Blackwell, Ph.D. (psychology); Thos. C. Blackwell, Ph.D. (spychology); Warren T. Brown, M.D. (psychiatrist); John Waterman, M.D. (psychiatrist and neurologist); H. E. Moore, Ph.D. (sociology); and Mrs. Beulah Temple Wild (representative of *Mental Health Society*).

[2] Members of this Advisory Council were as follows: Doctor R. Lee Clark, Jr., Director of M. D. Anderson Hospital and Surgeon in Chief; Doctor J. R. Ewalt, Dean, The Postgraduate School of Medicine and Psychiatrist; Doctor Robert L. Sutherland, Director, The Hogg Foundation for Mental Hygiene and Sociologist; and Doctor Carson McGuire, Head of Human Development, The University of Texas.

tasks represent lifetimes of work for many people of diverse training.

The major recommendation of the preliminary report was that an organized program for the utilization of psychologists in the total war against cancer within the institution be instigated. An integral part of that recommendation was the specification that adequate medical training courses, designed and developed to assist and enhance the insight and effectiveness of the psychologist, be offered.

The Program

Based upon recommendations of the exploratory report, an organized psychology program at M. D. Anderson Hospital was activated. The purpose of the program is a twofold one. First, it is the purpose to supplement medical efforts in the fight against cancer by contributing to the fundamental knowledge of the cancer patient as a person. This knowledge, combined with the skill of the psychologist and psychiatrist in alleviating emotional discomfort of people under stress, is premised to be of value to the physician in management and treatment of the cancer patient as well as of research importance. Second, it is the goal of the program to provide adequate medical training and supervised research experience to prepare psychologists for careers in medical-psychological research.

Administratively, the psychology program is under the direction, and an integral part, of the Department of Medicine. Functionally and professionally, it operates under the guidance of an inter-disciplinary Advisory Council. Members of this group are drawn from the disciplines of medicine, psychiatry, psychology, and sociology.[3] This body is responsible only to the Director of

[3] Activation of this group made of the program a cooperative endeavor between three divisions of The University of Texas: M. D. Anderson Hospital, The Hogg Foundation for Mental Hygiene, and the Graduate School of the Main Campus. Members of the present Advisory Council are: R. Lee Clark, Jr., M.D., Director, M. D. Anderson Hospital and Surgeon in Chief; Robert L. Sutherland, Ph.D., Director, The Hogg Foundation for Mental Hygiene and Sociologist; C. D. Howe, M.D., Chief of Medicine; J. B. Trunnell, M.D., Head, Experimental Medicine Section; Karl M. Dallenbach, Ph.D., Chief Psychology Department; Philip Worchel, Ph.D., Head, Clinical Psychology; Carson McGuire, Ph.D., Head, Human Development; Bill L. Kell, Ph.D., Counseling and Guidance; and B. R. Payne, Ph.D., Experimental Psychologist, Aviation School of Medicine, San Antonio, Texas.

M. D. Anderson Hospital, and its members are appointed by him. The function of the Advisory Council is a versatile, policy making, recommending, and evaluative one.

Training fellowships at a doctoral level[4] are set up with an annual stipend of $3,000.00 for outstanding student psychologists. Applications for these fellowships are considered by the Advisory Council and recommendations for appointment go from that body to the Director of the Hospital for action. Research experience of the fellow is to be supervised jointly by the doctoral committee of the individual and the Advisory Council. The appointment is for two years to provide ample time for completion of dissertational research, medical training courses, and some clinical experience with chronically ill patients. It is expected that the appointees will achieve an understanding of medical-psychological research and skills in working with emotional problems of the chronically ill underlying a future career as a medical psychologist with an area of specialization.

The total psychology program, then, develops into a three-dimensional one. These dimensions are medical training, research experience, and service to chronically ill patients. All three blend and interact, but each is a planned entity with a definite purpose and goal.

The medical training program. In setting up a medical training program for psychologists working in research and/or service in a medical setting, it was recognized that a transient period of exploration and evolution wherein a clarification of needs might emerge for incorporation into a permanent curriculum was necessary. The original tentative plan called for a two-year training program, combining courses in medical education with a research assignment and service experience with cancer patients. This temporary program was activated in September of 1952.

The first year courses consisted of Medical Orientation to Cancer by Types and Treatments, Anatomy of the Central Nervous System, and Endocrinology I. Medical Orientation to Cancer by Types and Treatments consisted of a series of 16 lectures conducted by members of the M. D. Anderson Hospital Staff.

[4] Candidates are said to be at a doctoral level when all course work for a Ph.D. has been completed, all internships regularly required consummated, and only dissertational research remains to be done.

The problem of cancer was presented from the standpoint of theory, site of the neoplasm, and methods of treatment. The second course, Anatomy of the Central Nervous System, was conducted by the Chief of Pathology and his Assistant. This series of 16 lectures and laboratory sessions was designed to acquaint the psychologists with the structure and function of the central nervous system, emphasizing reactions to stress as well as normal functioning. Endocrinology I was organized in a manner to give further knowledge and some experience in laboratory experimentation in the field of basic endocrinology. It was taught by the Head of the Experimental Medicine Section.

During the first year as the medical courses progressed, suggestions for improvement and addition were presented. A valuable development came through a joint-sponsorship agreement between M. D. Anderson Hospital and The University of Texas Postgraduate School of Medicine. This agreement made official credit for the courses possible. Completion of the prescribed two years of medical training courses will now make the student eligible for a medical training certificate awarded by The Postgraduate School of Medicine.

Another important development enhanced the value of the originally planned medical curriculum for the second year. This addition came in the form of an offer from The University of Texas Medical Branch, Galveston, Texas, to the effect that the facility there was available to this new endeavor in any way that the organized offerings of that institution might contribute.

As a result of these two developments, the program has been made available to all psychology trainees in the Texas Medical Center at Houston, and to psychology fellows at the Medical Branch, Galveston, Texas. Consequently, psychology students at the Medical Branch are enrolled in Endocrinology II, driving to Houston weekly for a two-hour session. Taking advantage of the generous offer of the Medical Branch, psychologists of M. D. Anderson Hospital likewise are traveling to Galveston weekly to attend a course in the History of Psychiatry there. Cooperative efforts and productive participation on the part of members of the medical staff of each of these institutions constitute a demonstration of teamwork among three divisions of The Uni-

versity of Texas in organizing and developing a new field of educational endeavor.

Second year courses are at present under way. Endocrinology II will be completed on February 11. This course is to consist of 16 sessions of two hours each, devoted to a systematic study of The General Adaptation Syndrome. It is being taught by the Head of the Experimental Medicine Section, M. D. Anderson Hospital.

The second course of the second year offering will be Functional Neurology. The Staff Neurologist will teach the course, assisted by various other specialists from time to time. The course will consist of 16 lectures concerned with a functional study of the anatomy of the central nervous system with case presentations of the various diseases centering around this system.

The third course of the second year will be conducted as a workshop in psychosomatic implications in cancer. Guest lecturers from within the state and from several institutions outside Texas will be invited to participate in the formulation of a theory of psychosomatic implications in cancer. This workshop will extend over one week of operations.

Recent trends of evolution and development indicate further refinement and organization in the medical training program in the near future. Negotiations are under way to apply for accreditation of the courses through the Graduate Legislative Council of The University of Texas System by developing a minor in Medical Foundations for psychology and basic science doctoral students.

The research program. Like the medical training program, the research program has undergone refinements as the year progressed. Originally, research was instigated in two areas: behavior and physiochemical-psychological interaction.

The behavior studies have been concerned with psychological motivations behind delaying, detouring to non-medical sources, and refusing treatment when cancer is suspected. An exploration of anxiety engendered from the knowledge of cancer is considered a basic must in this research. To adequately probe this emotion, development of specific tools designed for the purpose is essential. In the course of investigations now under way,

psychological tools are being formulated for this study, and a pilot project in this area will be initiated in the near future.

Research in the physiological-psychological interaction category has centered around two specific problems. The first concerns an evaluation of psychological changes following administration of sex hormones to women with malignant tumors of the breast.[5] The second is a somewhat parallel study of men suffering from prostatic cancer. Both of these investigations are being done in conjunction with a giant metabolic study being done by the Section of Experimental Medicine. Numerous physiochemical assays are being run by the laboratories for comparison with psychological measures as a part of the integrated physiochemical-psychological interaction research.

As the research has progressed, numerous avenues of diverse investigations have developed. Consequently, at a recent meeting of staff and Advisory Council members,[6] a proposed expansion and organization of research interests was compiled. This proposal set forth four specific sections of research possibilities. (See Table 1.) First, psychophysiological basis for cancer included such items for exploration as predisposition, trigger mechanism, and organ selection. Second, Responses to Cancer included motivational modifications, alterations in personality structure, psychological effects of organic malfunction, overt behavior, and psychotherapeutic problems. Third, psychological responses to treatment would involve study of the effects of surgery, of chemotherapy, and of radiation therapy on the patient. And, fourth, the psychological role of the endocrine system was given attention as a specific section of research.

As in the medical training area, the research program is a flexible one. The policy is to keep an open mind to research possibilities, to reject no hypothesis presented by medical staff members without systematic investigation, and to broaden the research horizons as the needs and interests of the research group indicate.

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[5] John I. Wheeler, Jr., graduate student in clinical psychology, The University of Texas. This research will be completed and reported during 1954.

[6] Appreciation and credit is gratefully acknowledged to members of the Advisory Council, Carson McGuire, Philip Worchel, the Hospital Psychiatrist, Dorothy Cato, and the Psychology Fellow, John I. Wheeler, Jr., for guidance and active participation in the organization of possible research areas.

The service program. This area of the total program is under the supervision of the hospital psychiatrist. It is a referral service for physicians wishing psychological evaluation of individual patients. Diagnostic referrals are few, but there is much need

TABLE 1

Medical-psychological research approaches in cancer

General Area of Research	Specific Investigations Proposed
I. Psychophysiological Basis for Cancer	A. Predisposition B. Trigger Mechanism C. Organ Selection D. Other
II. Responses to Cancer	A. Motivational Modifications B. Alteration in Personality Structure C. Psychological Effects of Organic Malfunction D. Overt Behavior E. Psychotherapeutic Problems F. Other
III. Psychological Responses to Treatment	A. Effects of Surgery B. Effects of Chemotherapy C. Effects of Radiation Therapy D. Other
IV. The Psychological Role of the Endocrine System	

for supportive therapy with emotionally disturbed patients. This is the newest phase of the program and is still in organizational stages. Planning includes work in group therapy and individual psychotherapy on a research basis.

The Long-Range Plan

The psychology staff at present consists of a full-time research psychologist, a part-time psychiatrist, a psychology fellow, and a secretary. The long-range plan is to add to the program, in a cumulative fashion from year to year, other fellows in the various fields of psychology as research and service needs of the hospital dictate. Exploratory investigation is in progress to determine possible contributions to the effectiveness of the medical education program from the addition of a psychologist specializing in personnel consultation. This psychologist would be responsible for screening and evaluation of medical and scientific

residents training at M. D. Anderson Hospital. Preliminary evaluation of the service area of the program indicates a need for emphasis on research in group therapy and psychotherapy with cancer patients. Special emphasis is contemplated in the study of the correlation of physical and biochemical changes with alterations in hormonal and mental states of patients with cancer. The need for adequate research in the fields of family and culture suggests possibilities for contributions to be made from sociology and anthropology. The eventual goal is a full-time staff of a minimum of three psychologists representing various fields, three psychology fellows, a fellow in sociology, and one in anthropology. Such a basic staff would form an interdisciplinary research team in the social sciences working in conjunction with, and as members of, a medical research group.

Within the medical setting of a research hospital, a psychology program constructed upon the needs of that institution is taking shape. The history of the conception and development of the idea has been outlined. The administrative organization and the three dimensional training-research service functions of the program have been reviewed. Attention has been directed to the future goals of the project. The program is an ambitious one; only initial steps have been made. Much remains to be learned, for psychological research and service in a medical setting is almost uncharted. The initiation of such a program is an experiment in the team approach to understanding and treatment of patients. Progress to date indicates that such an approach may be successfully applied in a medical-research setting. Care is being taken to integrate the program into the existing framework of medical practice and hospital routine. The ready acceptance and use of the knowledge gained will be the criteria of the success of this objective.

A NEW AMERICAN HARPACTORINE GENUS

(Hemiptera, Reduviidae)

JOE C. ELKINS*

The genera of the American subfamily *Harpactorinae* are in need of critical reevaluations. The discovery of the genus described here is a result of some preliminary investigations of this nature and it is segregated from *Zelus* Fabr., the largest American harpactorine genus. This matter has practical importance since some harpactorine species may be involved in the transmission of Chagas' disease.

Among other American genera in the same subfamily, current investigations indicate that several genera should be synonymized. This has occurred in one instance (Elkins, 1954).

All species seen which belong to this new genus constitute a remarkably homogeneous group from the viewpoint of general external morphology; consequently, in this work the generalized specific descriptions are brief, but the genitalia are carefully described and figured, for with the exception of a few minor differences, the male and female genitalia are the only reliable features for specific differentiation. The scale accompanying the illustrations is in millimeters.

It is a pleasure to thank the following for the loan of material under their care: Dr. Max Beier, Naturhistorisches Museum (NM), Vienna, Austria; Dr. Henry Dietrich, Cornell University (COR), Ithaca, N. Y.; Dr. Kenneth Hayward, Fundacion Miguel Lillo (FML), Tucumán, Argentina; Dr. A. A. Pirán, Division de Zoología Agrícola, Ministerio de Agricultura (ZA), Buenos Aires, Argentina; Dr. Reece I. Sailer, United States National Museum (US), Washington, D. C.; R. P. Gregorio Williner S. J. (through Dr. Petr Wygodzinsky), Instituto de Historia Natural "Sanchez Labrador" (SL), San Miguel, Argentina; Dr. René Malaise, Naturhistoriska Riksmuseets (STOC), Stockholm, Sweden, for the loan of the types of *Zelus pallens* (H.–Sch.), *Zelus plebejus* Stål and *Zelus prolixus* Stål; Dr. Petr Wygodzinsky (WYG), Tucumán, Argentina, for the loan of material from his collection and that of the Instituto Medicina Regional (IMR), Tucumán, Argentina, and for valuable aid and advice.

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Atopozelus, n. gen.

Type species, *Atopozelus opsimus* n. sp. (fig. 1).

This genus has the following features in common with *Zelus* Fabr.: fore femur as long as, or longer than, hind femur; basal and distal membrane cells of hemelytron more or less subequal in length, one never much longer than the other; second rostral segment conspicuously longer than either basal or terminal segment. In general, it differs from typical *Zelus* by the small size and the head being abruptly constricted basally. In particular, it differs from the classical limits of *Zelus* by the constant feature of the basal rostral segment either attaining or surpassing the anterior margin of the eyes, the absence of claspers in the male, the lack of a true anterior bridge on the basal plate (figs. 11, 13), and the more robust, subtriangular mandibular lever (figs. 14, 15).

Perhaps most closely related to the subgenus *Zelus (Zelus)*, which is characterized by a spineless posterior pronotal lobe; however, a few individuals of *Atopozelus pallens* (H. Sch.) exhibit the condition of a minute salient at each humeral angle, but this condition does not approach that found in the subgenus *Pindus* Stål.

Since they are all small, generally pale stramineous insects, species of *Ttopozelus* might be confused with *Zelus prolixus* Stål, but the male of *Atopozelus* can easily be distinguished by the absence of claspers and both sexes by the basal rostral segment either attaining or surpassing the anterior margin of the eyes.

Small insects, varying from 7 mm to 11 mm, neither sex constantly larger than the other. Dull, varying from pale stramineous to brown, color pattern variable and generally nondistinctive. General shape oblong and narrow; pronotum, posteriorly, wider than abdomen which is not parallel sided, but tapering somewhat from the basal segment to apex. Hairs of head, body and corium very sparse, short and inconspicuous; hairs of femora erect; of fore tibiae, erect, dense and bristle-like; of mid and hind tibiae, erect, less dense and not bristle-like; of antennae, rather dense on third segment, inconspicuous on other segments.

Figure 1, *Atopozelus opsimus* n. sp., male; figure 2, male hypopygium, *A. scepanius* n. sp.; figure 3, male hypopygium, *A. omalus* n. sp.; figure 4, male hypopygium, *A. pallens* (H.–S.); figure 5, male hypopygium, *A. opsimus* n. sp.; figure 6, female external genitalia, *A. omalus;* figure 7, female external genitalia, *A. pallens;* figure 8, female external genitalia, *A. opsimus;* figure 9, aedeagus, *A. scepanius;* figure 10, aedeagus, *A. pallens.*

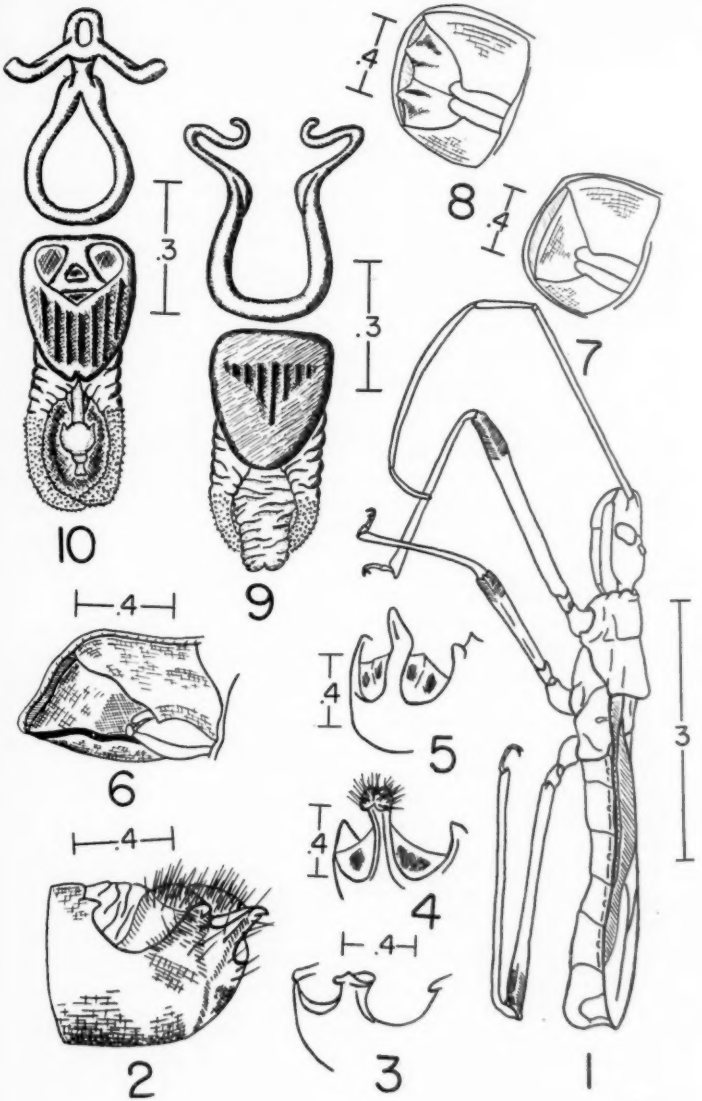

PLATE I

Head shorter than pronotum, not tapering to collum, but abruptly constricted basally. A rather shallow dorsal transverse interocular sulcus just behind middle of head. Clypeus conspicuous. Dorsal width of eye one-half or less width of interocular space at vertex; ocelli not greatly elevated, if at all; each ocellus located just behind interocular sulcus, the outer margin of ocellus approximately on line with inner margin of eye. Basal rostral segment either attaining or surpassing anterior margin of eyes; second segment either subequal to, or longer than, first and third combined. Antenna less than total length of insect, first segment a little longer than head and pronotum together, third segment slightly thickened in male. Mandibular lever subtriangular, lacking prolongations at apices of angles.

Pronotal collar narrow, bulbous at each lateral extremity. Pronotum constricted dorsally just before middle; anterior lobe smooth, with longitudinal median sulcus; posterior lobe rugulose and shallowly punctate. Mesothorax and metathorax simple; hairs of mesosternum and metasternum a little longer than on remainder of thorax. Scutellar length a little less than that of anterior pronotal lobe; apical process short and blunt, sometimes upturned.

Fore femora incrassate, a little longer than hind femora; mid femora shorter than preceding. Corium with hairs very short, sparse and appressed. Cubital quadrangular cell of hemelytron rather narrow; length and width of basal membrane cell slightly more than, or subequal to, length and width of distal cell (fig. 16); apical angle of distal membrane cell not reaching apex of stigma. Abdomen narrow, with a few inconspicuous hairs. Laterally, each spiracle approximately at middle of each connexival segment. Claspers absent in male; anterior bridge of basal plate missing.

Generic name from Greek ἀτόπος, strange, out of the way. + *Zelus*, the etymology of which is unknown to me.

KEY TO THE SPECIES

1—Head dorsally and laterally, pronotum dorsally, scutellum and hami, brown; second antennal segment black for a little more than distal half; median longitudinal sulcus of anterior pronotal lobe rather deep; posterior process of male hypopygium bifid or U-shaped apically, apex of each lateral point in the form of a small spur (fig. 2) ..... *scepanius* n. sp.

—Head and anterior pronotal lobe generally pale stramineous; second antennal segment stramineous or brown; median longitudinal sulcus of anterior pronotal lobe rather shallow; posterior process of male hypopygium not bifid apically 2

2—Posterior process of male hypopygium not reaching level of lateral hypopygial border, process somewhat flattened and lip-like apically (fig. 3); female IXth tergite sub-horizontal (fig. 6) ..... *omalus* n.sp.

—Posterior process of male hypopygium reaching level of lateral hypopygial border, either bulbous or a little pointed apically; female IXth tergite sub-vertical 3

3—Posterior process of male hypopygium bulbous and densely hairy apically (fig. 4); IXth tergite of female simple (fig. 7) ..... *pallens* (H.–Sch.)

—Posterior process of male hypopygium simple, somewhat pointed apically and not covered with dense hairs (fig. 5); IXth tergite of female with two dorso-lateral prominences (fig. 8).....*opsimus* n. sp.

Atopozelus scepanius n. sp.

This species does not fit closely into the compact group which is comprised of the other three species. It is generally darker with consistently darkly annulated legs, and the anterior pronotal lobe has a deeper longitudinal median sulcus. The bifid or U-shaped posterior process of the male hypopygium, coupled with the anteriorly unfused basal plate suggests the possibility of a separate subgeneric status, but due to the lack of females and other insufficient data, the erection of a subgenus cannot be justified currently.

Length 8.5 mm to 8.7 mm. Antennae with three dark brown annuli on basal segment; second segment black for a little more than distal half; third and fourth segments concolorous dark brown. Head laterally and dorsolaterally light brown, a pale transverse median stripe the dorsal length; head ventrally, and rostrum, stramineous. Ocelli rather large and slightly elevated. Pronotum dark brown; longitudinal median sulcus of anterior lobe rather deep and widely trenched at top. Laterally, thorax and abdomen stramineous, with widely scattered brown spots. Hami dark brown, a wide dusky stripe the median length of hemelytra. Femur with three brown annuli, the basal annulus faint in some individuals; tibiae with four brown annuli.

Male genitalia. Superior posterior border of hypopygium with wide median process that is bifid or U-shaped apically (fig. 2), apex of each lateral point in the form of a small spur; behind bifid apex two dorsal pads covered with dense long hair tufts; entire median process covered with long hairs. Basal plate separated distally, apical lateral arms slender and curved inward (fig. 9); basally, basal plate fused and thick. Phallosoma thickly rugulose, with a dorsal flap covering most of organ when unextended; endosoma lacking distinctive structures.

Trivial name from Greek σκηπάνιον, τό, a scepter.

Material examined. Holotype, BRAZIL, Rio Verde, Goiaz, 1–1944, J. C. Carvalho, ♂, temporarily in my collection. Paratypes, BRAZIL, same data as holotype, 2♂♂, ELK; Campinas, Goyas, 1–9–36, Borgmeier et Lopes, 1♂, WYG.

Atopozelus pallens (Herrich-Schaeffer, 1848) *comb. nov.*

Euagoras pallens Herrich-Schaeffer, 1848 Wans. Ins. *8*:46, fig. 819; Stål, 1872, K. Vet.-Ak. Hdl. *14*(4):89; Champion, 1898-1902, Biol. Cent. Am., Ins. Hem.-Het., Rhynch. *2*:256.

Zelus pallens (Herrich-Schaeffer). Stal, 1872, loc. cit. :89; Fracker, 1913, Proc. Iowa. Ac. Sci. *19*:239; Wygodzinsky, 1949, Mon. Inst. Med. Reg., Tucumán *1*:50.

Zelus [?] *pallens* (Herrich-Schaeffer). Champion, 1898-1902, loc. cit. :256.

Zelus plebejus Stål, 1860, K. Vet.-Ak. Hdl. *2*(7):74; Stål, 1872, loc. cit. :89; Wygodzinsky, 1949, loc. cit. :50. *(syn. nov.)*.

Closely resembling *Atopozelus opsimus* n. sp. and *Atopozelus omalus* n. sp., but the posterior median process of the male hypopygium is longer than that of *A. omalus* and directed back at a wider angle than either of the other two species; also, it is apically bulbous and covered with long hairs. The IXth tergite of the female flattened or slightly concave and sub-vertical, whereas in *A. opsimus* the IXth tergite has two pointed or rounded dorso-lateral protuberances, and in *A. omalus* this tergite is relatively longer and sub-horizontal. All specimens seen of *A. omalus* and *A. opsimus* have a red annulus at the apex of each femur, but this condition is variable in *A. pallens*, some individuals having totally pale stramineous legs and bodies. In a few individuals there is an inconspicuous minute salient at each pronotal humeral angle; this condition was not observed among specimens of the other species.

Male genitalia. Posterior median process of hypopygium long and slender, attaining level of lateral hypopygial border; apex bulbous and covered with dense hairs. Basal plate (fig. 10) fused beyond apical half; lateral apical arms long and slender, slightly curved back at apices; proximal half of basal plate abruptly split and ring-like, fused at base. Phallosoma with a triangular chitinous structure basally, from which extends a dorsal flap that covers most of organ when unextended; endosoma with a bulbous conformity distally; surface of surrounding phallosoma rough due to many small spurs.

Female genitalia. Ninth tergite sub-vertical and usually slightly concave, sometimes flattened; posterior gonopophyses exposed distally; anterior gonopophyses horizontal, not covered by lobes of VIIIth sternite.

Figure 11, aedeagus, *A. opsimus;* figure 12, aedeagus, *A. omalus;* figure 13, aedeagus, *Zelus longipes* (Linné); figure 14, mandibular lever, *A. opsimus;* figure 15, mandibular lever, *Z longipes;* figure 16, fore wing, *A. opsimus;* figure 17, hind wing, *A. opsimus;* figure 18, egg cluster, *A. omalus;* figure 19, freshly emerged nymph, *A. omalus*.

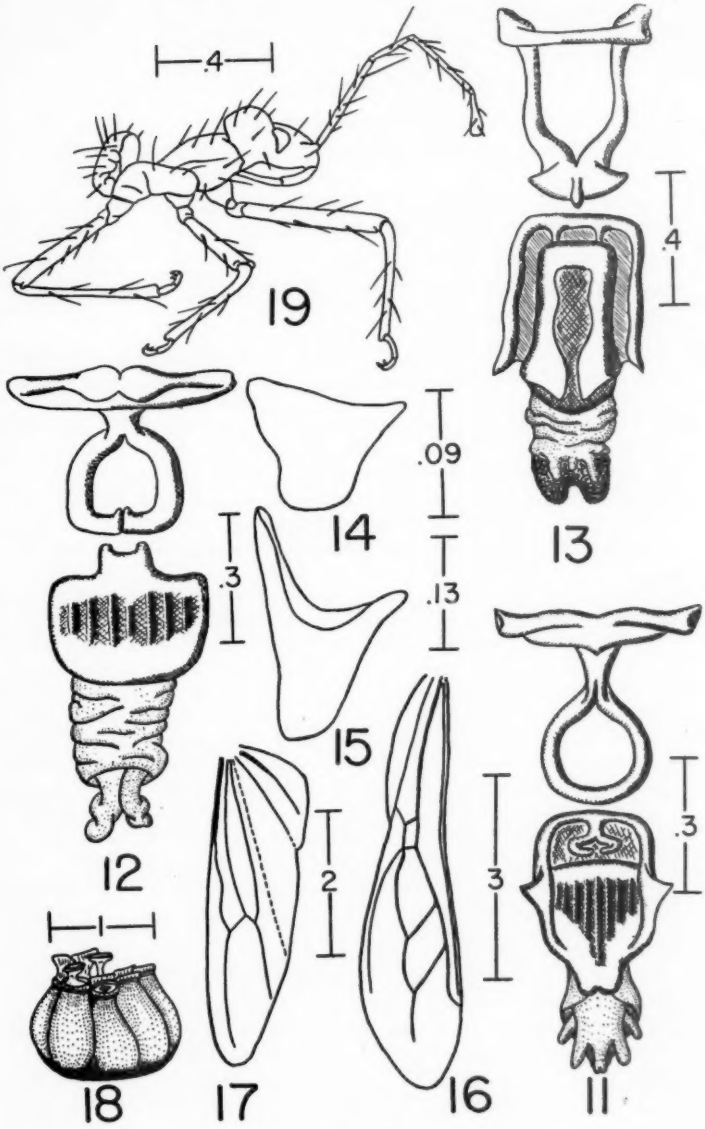

PLATE II

Material examined. Holotype, STOC, ♀, bearing following labels: Brasil, Typus, *Zelus pallens* H.–Sch.; 3 ♂♂ (including type of *Zelus plebejus* Stål); 8 ♀♀.

Distribution. Brazil, ?Mexico (Champion, 1898–1902), British W. I., Venezuela.

Atopozelus opsimus n. sp.

Resembling *A. pallens* as mentioned previously; also, close to *A. omalus*, but the posterior median process of the male hypopygium is longer and not flattened apically. The IXth female tergite of *A. omalus* lacks the dorso-lateral protuberances and is more sub-horizontal.

Length 9.2 mm to 10.5 mm. Color varying from generally pale stramineous to brownish on posterior pronotal disc, scutellum and hami; hemelytra in some individuals with wide dusky stripe along entire median length; all femora with red annulus at apex, a narrow brown ring immediately beneath red annulus in a few specimens; tibiae concolorous or each with one or two sub-basal brown annuli.

Ocelli slightly raised or not at all. Anterior pronotal lobe with shallow narrowly trenched longitudinal median sulcus.

Male genitalia. Superior posterior border of hypopygium deeply emarginate on either side of posterior median process (fig. 5); the latter long, angling slightly back along apical ⅓ to ¼ and somewhat spear-shaped at apex, rather slender in some individuals, noticeably broadened in others, without conspicuous pilosity. Basal plate (fig. 11) fused along apical half, from which arises two apical lateral arms which are thick and long; basal half of plate split, widely separated and ring-shaped, fused at extreme base. Phallosoma with Y-shaped structure basally, from which extends a dorsal flap which covers most of organ when unextended; endosoma with five tentacle-like processes distally.

Female genitalia. Ninth tergite sub-vertical, with two pointed or rounded protuberances dorso-laterally; posterior gonopophyses exposed distally; anterior gonopophyses horizontal not covered by lobes of VIIIth tergite.

Trivial name from Greek ὄψιμος, tardy, late of fulfillment.

Material examined. Holotype, PARAGUAY, Horqueta, 57—10, W 23—24 N, 44 kilm. E. Paraguay River, 4–12–33, Alberto Schultze, ♂, temporarily in my collection. Paratypes, PERU, Tingo Maria, 630m, 7–1949, 4K4 1238, Weyrauch, 3♂♂, 1♀, ELK, 1♂, WYG; BOLIVIA, Sur Yungas, Chulumani, 148, Bridaroli, 2 ♂♂, SL; PARAGUAY, San Bernardino, Fiebrig, 2 ♂♂, 4♀♀, NM; Horqueta, 57—10, W. 23&24 N, 44 kilm. E. Paraguay River, 1–27–36, Alberto Schultze, 2 ♂♂, ELK; BRAZIL, Nova Friburgo, 8–10–45, P. Wygodzinsky, 1♀, WYG; Pirapora, Minas Gerais, 11–13–19, Cornell Univ. Exp., Lot 569, Sub. 98, 1♂, COR; S. Paulo, 3–49, E. J. Hambleton, 1♂, 1♀, US; M. Gerais, E. J. Hambleton, 1♀, US; Nova Teutonia, Santa Catarina, 27° 11′—52° 23′ L., 9–16–44, F. Plaumann, 1♂, WYG; C. R.

Claro, 9–47, J. C. M. Carvalho, 1 ♂, WYG; Carmo do Rio Claro, Sul de Minas, J. C. M. Carvalho, 1 ♂, ELK.

Atopozelus omalus n. sp.

Similar to *A. pallens* and *A. opsimus* as previously stated. This is the smallest species of the genus and generally has less incrassate fore femora. Some individuals have darkly annulated legs, but this is a variable feature.

A female from Urundel, Argentina, is accompanied by a legume leaf on which are two clusters of eggs and a freshly emerged nymph. The eggs are in close clusters, cemented tightly together. The egg is similar to that of *Zelus socius* (Uhler) as figured by Readio (1929), but broader at the base (fig. 18). The nymph is figured (fig. 19).

Length 7 mm to 8.5 mm. Antennae, head, rostrum and body totally pale stramineous in a few individuals, posterior lobe of head and antennae brown in others; posterior pronotal lobe, scutellum, hami and corium brown in the majority of specimens seen; each femur with a red annulus at apex, in some individuals one to two brown annuli sub-apically; each tibia concolorous pale stramineous in some, brown concolorous or with two brown sub-basal annuli in others.

Ocelli noticeably raised in most individuals, slightly elevated or not at all in a few. Anterior pronotal lobe with a shallow, narrowly trenched longitudinal median sulcus. Fore femora slightly incrassate.

Male genitalia. Posterior median process of hypopygium rather broad and short, which is flattened and lip-like apically, the apex not attaining the level of the lateral hypopygial border. Basal plate fused over apical ⅓ (fig. 12), from which arise two lateral arms that are short and narrow; basal ⅔ split apart and ring-like, fused at extreme base. A dorsal flap covering most of phallosoma when unextended; endosoma with two bulbous processes distally.

Female genitalia. Ninth tergite (fig. 6) sub-horizantal and slightly concave, resulting in the posterior gonopophyses being noticeably exposed dorsally; anterior gonopophyses horizontal, not covered by lobes of VIIIth sternite.

Trivial name from Greek ὅμαλος, of like degree, average.

Material examined. Holotype, ARGENTINA, Santa Fe, 11–27–30, S. J. Bridaroli, ♂, SL. Paratypes, ARGENTINA, same data as holotype, 1 ♂, SL; Santa Fe, 2–11–29, S. J. Bridaroli, 1 ♀, SL; Portezuelo, Prov. Catarmarco, 8–14–38, A. O., 1 ♂, WYG; Guayacolac, Formosa, 2–49, 1 ♂, IMR; Urundel, Salta, 11–30–48, Wygodzinsky y Monrós, 1 ♀, IMR; Salta, 6–29–44, 1 ♂, FML; Las Brenas, Prov. Pres. Perón, 7–10–49, Pirán, 2 ♂ ♂, 1 ♀, ZA; Capiasuti, Salta, 8–5–44, 1 ♀, FML; Tucumán, City, 4–24–46, Abalos, 1 ♀, IMR; BRAZIL, Nova Friburgo, 900m, 8–10–45, P. Wygodzinsky, 1 ♀, ELK.

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Elkins, J. C.: A Synopsis of *Atrachelus* (Hemiptera–Reduviidae). To appear in 1954, *Proc. Wash. Ent. Soc.*

Readio, P. A.: Studies on the biology of the Reduviidae of America north of Mexico, *Univ. Kans. Sci. Bull.*, 17(1): 5–291, 21 pls., 1927.

Summary. *Atopozelus* n. gen. is segregated from *Zelus* Fabr. Four species are included in the new genus as follows: *Atopozelus pallens* (Herrich-Schaeffer), *Atopozelus scepanius* n. sp.; *Atopozelus omalus* n. sp. and *Atopozelus opsimus* n. sp. This reclassification may be significant in relation to factors involved in the transmission of Chagas disease.

MEASUREMENTS OF HOLOTYPES

(Unless otherwise specified, millimeter measurements in length only)

	A. opsimus mm	A. omalus mm	A. scepanius mm
Total length	9.20	7.22	8.70
Head	1.78	1.50	1.60
Eye	.40	.35	.40
Width of head at eyes	.92	.80	.90
Width of eye	.29	.20	.30
Anterior lobe of head	.95	.70	.70
Posterior lobe of head	.83	.80	.90
Basal antennal segment	3.44	3.42	3.35
2nd antennal segment	1.10	1.00	.98
3rd antennal segment	3.70	2.83	2.91
4th antennal segment	.82	...	.71
Basal rostral segment	.71	.60	.55
2nd rostral segment	.90	.87	.91
3rd rostral segment	.35	.30	.32
Thorax	1.95	1.49	1.71
Anterior thoracic lobe	.85	.71	.71
Posterior thoracic lobe	1.10	.78	1.00
Anterior thoracic lobe width	1.11	1.00	1.20
Posterior thoracic lobe width	1.81	1.58	2.11
Fore coxa	.55	.41	.45
Fore femur	3.60	3.10	3.59
Width of fore femur	.40	.25	.39
Fore tibia	3.81	3.20	3.95
Fore tarsus	.42	.20	.39
Mid coxa	.59	.39	.53
Mid femur	2.50	2.49	2.15
Mid tibia	3.11	2.51	2.62
Mid tarsus	.45	.42	.45
Hind coxa	.61	.41	.50
Hind femur	3.39	3.12	2.89
Hind tibia	4.10	3.50	3.49
Hind tarsus	.50	.42	.49
Abdomen	4.90	3.49	4.55
Width of abdomen at base	1.35	1.35	1.40
Fore wing	5.89	4.83	5.55
Hind wing	4.52	3.90	4.13

STUDIES ON GASTRIC MUCIN

I. In Vivo and In Vitro Studies with Shigella dysenteriae*

Frank B. Engley, Jr.

Various theories have been advanced relative to the mode of action of gastric mucin as a virulence enhancing agent or rather a resistance lowering substance. A review of the literature on the subject (Olitzki, 1948) suggests the following theories. It enhances the virulence of the organism, acts as a culture medium supplying nutrients and growth factors to the organism, lowers the resistance of the animal to infection by the toxicity of substances in the mucin, destroys complement thus preventing lysis of the cells, coats the cells with a covering protective against the host defenses, inhibits antibody obsorption by the organisms, inhibits the appearance of leukocytes, inhibits phagocytosis and/or intracellular digestion of bacteria by fixed and mobile cells, increases the permeability of the barrier between the abdominal cavity and the blood stream and neutralizes or inhibits the bactericidal action of blood and body fluids. Studies reported here with *Shigella dysenteriae* suggest the latter mode of action as one of the important methods by which gastric mucin is able to aid organisms in invading the host, causing infection and subsequent death of the animal.

The antibacterial action of whole blood and its various cellular and humoral components have been the study of various workers and the overall problem is discussed quite completely in immunology and bacteriology texts such as Topley and Wilson (1946).Briefly, it has been shown that the leukocytes besides being phagocytic, produce leukins and the platelets form plakins, both of which have antibacterial activity. The erythrocytes apparently do not have antibacterial activity in the form and in the concentration in which they are present in the blood stream. The fluid portion of the blood contains the heat labile complement which is active in aiding phagocyosis as well as lysis of susceptible bacterial cells in conjunction with antibody of the globulin

* From the Department of Bacteriology and Parasitology, The University of Texas Medical Branch, Galveston. Based in part on work conducted at Biological Laboratories. Camp Detrick, Frederick, Maryland. Received for publication November 27, 1953.

fraction. Also present in the serum or plasma are heat stable antibacterial substances which can act without complement. These substances have been termed β (beta) lysins and are not well defined or completely understood. In studying the antibacterial action of blood and body fluids then, we find that there is a series of complex mechanisms of cellular and humoral and even cellular-humoral combinations involved in the protection of the host from invading organisms.

Experimental data reported here are concerned with: (1) the action of gastric mucin in reducing the number of viable cells of *Shigella dysenteriae* necessary to cause fatal infections after intraperitoneal injection in mice (2) the *in vitro* antibacterial action of normal serum and body fluids for *S. dysenteriae*, and (3) the inhibition of antibacterial action of normal serum by gastric mucin. These have been the subject of preliminary reports (Engley, 1950, 1952, 1953).

Experimental

During a series of experiments to standardize an infection of *S. dysenteriae* in experimental animals for prophylactic and therapeutic studies, gastric mucin was utilized as a resistance lowering substance. White Swiss mice (20 gram) were the test animals. The gastric mucin (Wilson 1701 W) was made up as a 5% aqueous preparation and sterilized by autoclaving at 15 lbs. pressure (121C) for 10 minutes. The pH of the original preparation was approximately 5.5. This was brought to pH 7.0 by the addition of sterile N/1 NaOH under aseptic conditions. Sterility controls were run in broth and by streaking over the surface of an agar plate. Toxicity controls were carried out in mice by the injection of 1 ml quantities intraperitorneally (IP) into six mice. These were observed closely over a 24 hour period and held for 14 days before discarding. The mucin was kept at 4C until used. After the 24 hour observation period, the mucin was utilized as a suspending medium for titration of infectivity of the test organism.

Ten-fold serial dilutions ($10^{-1} - 10^{-9}$) of a young (18 hour) broth culture of the test organism were prepared in nutrient broth and 1 ml of each was transferred to a tube containing 9 ml of sterile 5% gastric mucin and mixed thoroughly. This procedure gave more accurate dilutions than making the serial dilu-

tions through mucin because of the high viscosity of the gastric mucin. Six mice were then injected IP with 1 ml aliquot amounts from each of the serial dilutions and observed for deaths over a 14 day period. At the same time as the injection of the mice, pour plates were made from the broth dilutions and viable counts were determined after 48 hours of incubation at 37C. The majority of deaths occurred in the first 24 hours after injection with some deaths in the higher dilutions being delayed until 30–48 hours and occasionally longer. A number of the mice from the higher dilutions were autopsied upon death and the heart's blood was streaked on agar plates for re-isolation of the *S. dysenteriae* organisms.

It was observed that in changing to various lots of mucin (Wilson 1701 W) that some quantitative differences were obtained in the level of infectivity of the organisms for mice. To further evaluate this, a number of lots of mucin were obtained.* The resulting data from tests with these lots of mucin are shown in Figure 1. Seven different lots were available and were tested with one strain of *S. dysenteriae* (14–4). The quantitative differences are quite striking. The control mice were injected with dilutions of the culture in broth with infectivity at only a dilution of $10^{-1.5}$ or approximately 10^8 organisms necessary to kill 50% of the mice (LD_{50}). With the mucins as the suspending medium as increase in infectivity in all cases was noted. With five lots the increase was only 50–100 fold (10^2). With one (68125), the increase was in order of 100,000 or 10^5 fold while with another lot (57735), the increase was as high as 10 million or 10^7 fold. In the latter case, approximately only 10 viable cells were necessary for infection and an LD_{50} in the test mice. These quantitative data indicate the importance of differences in various lots of mucin for increasing infectivity. This has been noted previously with several different organisms (Nungester 1936, Olitzki, 1945, Sindbjerg-Hansen, 1943).

Another series of experiments with one lot of mucin (57735) and a series of *S. dysenteriae* strains bring out other facts concerning the interacting roles of the mucin and the organism in causing infection in the experimental animal. The data are shown graphically in Figure 2. The experimental procedure was

* Through courtesy of Dr. Daivd Klein of Wilson Laboratories.

similar to the previously described experiments. In this case, however, ten different strains of the test organism were available to be tested. The 14- and 43-series were obtained from the Army Medical School, the P-series was from the University of Pennsylvania and the 2308 R and S cultures were supplied by Dr. Rene Dubos of the Rockefeller Foundation. The LD_{50} values for each of the strains injected without mucin was in the same range. Only a dilution of $10^{-1} - 10^{-2}$ of the original culture containing approximately 5×10^{9} viable organisms was able to kill 50% of the test mice. With mucin as a suspending medium, a different picture

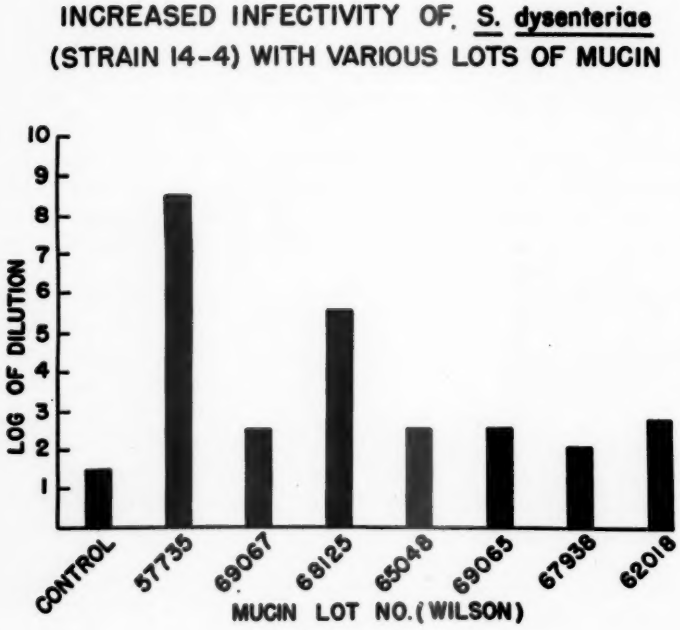

FIGURE 1

was seen. Each strain was increased in its ability to kill the mice but at somewhat different levels. Several strains were only increased 100–1000 fold (P52A, 43-A-17 and 43-A-14). Two strains (14–5 and 43-A-15) increased 10^{4} or 10,000 fold, while two others (P42 and 2308S) were able to infect at 10^{-5} or 100,000

fold dilution. The 14–2 strain increased 10^6 fold, while the 14–4 strain was able to infect at a dilution of $10^{-8.5}$. These results reveal that while the mucin may act upon the host in reducing its resistance to infection, the organism injected must have some virulence is reflected in the results shown here. The mucin must reduce some of the host resistance but not all and then the infecting organism is able to overcome the remainder depending upon its own capabilities. One culture, 2308R was a rough (R) culture in all respects. The ability to kill mice was raised slightly by the mucin. By definition it was avirulent. This increased killing power may have been merely an enhanced toxicity of the cells in the presence of mucin which has some toxicity itself.

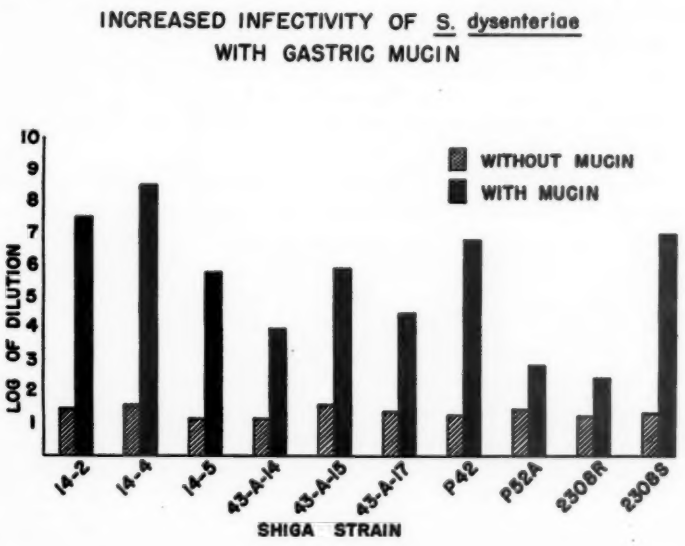

FIGURE 2

Similar studies made with a different lot of mucin (68125) and the same strains of *S. dysenteriae* gave a similar pattern of relative infectivity of these strains for mice but each at a slightly lower level. Such results might be expected from the data in Figure 1. All of these studies on varying the lots of mucin and varying the strains were carried out in one test animal. It might

be expected if guinea pigs or rats or perhaps another type of mouse other than the white Swiss mouse was used somewhat different patterns of infectivity might be obtained. Such tests were not performed here but are worthy of further investigation.

Efforts were made to determine the effect of the body fluids of the mice on the growth of *S. dysenteriae* organisms *in vitro*. It had been noted that peritoneal fluid of mice was bactericidal for *H. influenzae* (Fothergill, 1937) and blood was active against gonococci (Keefer 1938) meningococci (McLeod, 1941) and *Enterobacteriaceae* (Olitzki, 1945) (Sindbjerg-Hansen, 1943). Blood was obtained by cardiac puncture from mice and pooled for test. Some was allowed to clot. Another portion was defibrinated and the serum and cells separated. The cells were washed and added to an equal volume of saline for resuspension. One portion of serum was heated at 56C for 30 minutes to inactivate complement. Peritoneal fluid was obtained from mice following injection of 0.5 ml. of physiological saline IP and then fluid removed by needle and syringe 1–2 hours later. Peritoneal fluids from several mice were pooled for test.

The antibacterial activity of the body fluids and blood cells was determined by the addition of 10^5 (100,000) *S. dysenteriae* cells in 0.5 ml. amounts of broth to 0.5 ml. aliquot samples of each fluid and incubated at 37C. At intervals up to 24 hours after inoculation, samples were removed by standard loop and streaked on segments of nutrient agar plates to determine the presence and relative numbers of viable organisms. Summary data are shown in the first column of Table 1. It was found that whole mouse

TABLE 1

The Antibacterial Action of Mouse Blood Components and Peritoneal Fluid for S. dysenteriae and its Inactivation by Gastric Mucin*

Material	Antibacterial activity	Antibacterial activity with Mucin added
whole blood	+	—
cellular components	—	—
plasma	+	—
serum	+	—
serum (heated)	+	—
peritoneal fluid	+	—
broth control	—	—

+ = present
— = absent
* 10^5 viable cells added per ml.

blood, plasma, serum and peritoneal fluid all destroyed the organisms. The heated serum was also as active as the whole blood and unheated serum in destroying the bacteria, indicating that complement and complement mediated mechanisms were not the complete or major method by which the organisms were destroyed in the mouse blood. These findings suggest that the activity resides in the beta lysins of the serum. The active factor or factors present in the peritoneal fluid are not known but tests indicated that heated fluid had residual antibacterial activity in further tests suggesting that similar beta lysins might be present in peritoneal fluid.

Further studies with this same phenomenon were suggested by previous work cited with other organisms and species of animals (Olitzki, 1948) and by these findings. The effect of gastric mucin on the antibacterial action of body fluids was determined. Tests were set up similar to those just previously described but this time the 10^5 organisms were added in 0.5 ml. amounts of sterile 5% gastric mucin instead of broth. Incubation at 37C followed with samples being removed at intervals over a 24 hour period for plating on agar. A summary of the results are presented in the second column of Table 1. It was observed that the addition of gastric mucin allowed the *S. dysenteriae* cells to grow in all of the fluids under test. The blood, plasma, serum and peritoneal fluid which had antibacterial activity no longer destroyed the organisms when mucin was added. Mucin then is able to neutralize, inactivate or inhibit the antibacterial action of the body fluids of the mice.

Experiments were designed to determine whether sera from other species would have the same antibacterial activity against *S. dysenteriae*. Table 2 contains the resulting information from

TABLE 2

Inhibition of Normal Serum Bactericidal Activity by Gastric Mucin

Normal serum	Antibacterial activity	Bactericidal activity with Mucin present
Mouse	+	—
Rabbit	+	—
Sheep	+	—
Bovine	+	—
Human	+	—

— = no activity
+ = antibacterial activity present

these studies. The experiments were carried out similar to those described above for mouse serum where 10^5 viable organisms were added in 0.5 ml. of broth to sera of mouse, rabbit, sheep, cow and human origin. The first column of results in Table 2 shows that all sera tested were active against *S. dysenteriae*. When the viable cells were added to the sera in gastric mucin as a suspending medium rather than in broth, the antibacterial action was inhibited as shown in the second column of results in Table 2. These findings revealed that this antibacterial activity was not unique to the mouse but was also present in the serum of normal animals presumably not previously exposed to *S. dysenteriae* in nature. This statement is probably true in that this organism is not naturally infecting organism of animals other than man and is not found in their environment to stimulate natural immunity. The *S. dysenteriae* is found frequently in sections of the world other than this locality and the sera tested was from individuals not exposed to the organism in the laboratory.

Some precise quantitative determinations were made on the antibacterial action of normal rabbit serum and serum plus gastric mucin. Various ratios of serum and broth were prepared and it was found that several-fold dilutions of the serum could be made with broth without materially affecting the overall antibacterial activity. Figure 3 presents graphically the resulting curves of increase and decrease of viable *S. dysenteriae* in one of these experiments using serum and serum plus mucin where the ratio was serum to mucin at 5:1 with the control of serum to broth at a ratio of 5:1. These quantitative experiments were carried out as described. One ml. of sterile 5% gastric mucin was added to 5 ml. of normal rabbit serum which had been heat inactivated at 56C for 30 minutes. As a control one ml. of nutrient broth was added to another 5 ml. sample of rabbit serum treated in the same manner. The organisms were added to each sample in a 50 ml. Erlenmeyer flask in the number of 10^5 per ml. Incubation was carried out at 37C on a shaking machine having 100 oscillations per minute. The shaking assured thorough mixing of the organisms with the serum or serum-mucin combination. At 0, 6, 12, 18 and 24 hours following innoculation, samples were withdrawn, dilutions prepared and samples of each dilution placed in petri dishes for agar pour-plate counts. After 48 hours of incubation, colony counts were made on these plates to determine the num-

ber of viable *S. dysenteriae* organisms at the varying times. The data are plotted in Figure 3. In the serum, the original count of 10^5 per ml. dropped to 10^3 after 6 hours of incubation and no viable cells were found at 12 hours. In the serum-mucin mixtures, the viable count had increased at 6 hours to 10^7 organisms per ml. At 12 hours, the viable count approximated 10^9 cells per ml. at which level it remained through the 18 and 24 hour sampling periods. Thus it is shown that the antibacterial action of normal serum is able to destroy 10^5 *S. dysenteriae* cells within 12 hours at 37C, even when the complement has been inactivated by heat. In the presence of gastric mucin the cells are not killed but remain viable and increase in number quite rapidly.

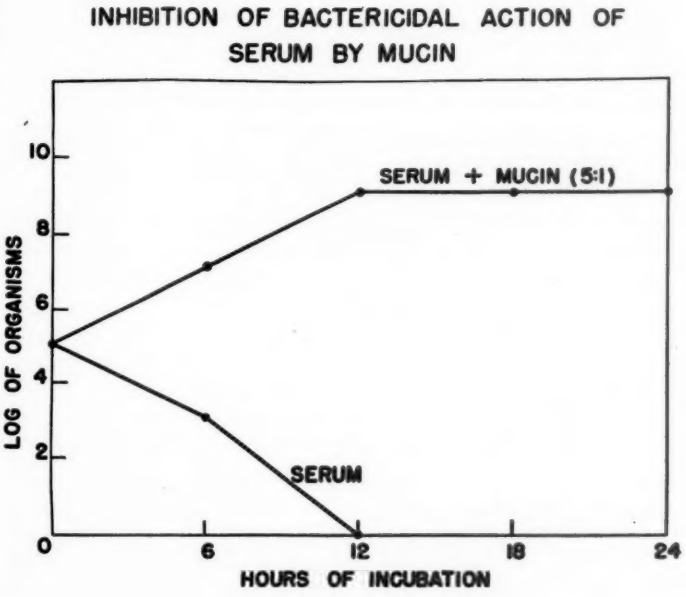

FIGURE 3

Attempts were made to determine whether or not the antibacterial activity might be associated with a particular fraction of the serum or plasma. Bovine plasma fractions were obtained from

Armour and Co.* (see Table 3) and human plasma fractions were obtained from Cutter Laboratories* (see Table 4). Each

TABLE 3

Bovine Plasma Fractions Tested for Antibacterial Activity

Fraction	Major components	Bactericidal activity
I	Fibrinogen	—
II	Gamma Globulin	—
III–1	Isoagglutinins	—
III–2	Prothrombin	—
IV–1	Alpha Globulin, Lipoprotein	—
IV–3,4	Alpha Globulin, some Beta Globulin and Albumin	—
Fractions Recombined		—

— = no activity

TABLE 4

Human Plasma Fractions Tested for Antibacterial Activity

Major fraction	Major components	Bactericidal activity
I	Fibrinogen	—
II	Gamma Globulin	—
III	Plasminogen, Prothrombin, Isoagglutinins	—
IV	Alpha Globulin, Lipoprotein, Albumin	—
V	Albumin	—
Other Fractions Tested		
II + III		—
II + III w		—
III — 1		—
III — 2		—
III — 2,3		—
IV — 1		—
IV — 4		—
IV — 6		—
IV — 7		—
V (Recryst)		—
Fractions Recombined (I–V)		—

— = no activity

fraction preparation was made up according to the concentrations normally present in whole plasma. One preparation was made for each plasma by recombining all fractions to original

* We are indebted to Armour and Co. and Cutter Laboratories for their generous supplies of these plasma fractions.

volume. All tubes were heated at 56C for 30 minutes. The test organism *S. dysenteriae* was added in the concentration of 10^5 viable cells in 0.5 ml. of broth to 0.5 ml. of the plasma fractions as in previous experiments with whole plasma and blood. Samples taken during incubation at 37C over a 24 hour period revealed an increase in the viable cell count in all samples indicating that the antibacterial action was not present in the bovine or human plasma fractions as tested (Tables 3 and 4). These findings suggested that the activity was lost during the fractionation process or on storage following fractionation since the recombined fractions in each case were negative for antibacterial action as well.

Other studies were made with mucin, mucin-like substances and unrelated compounds to determine whether other substances had similar activity in neutralizing the antibacterial activity *in vitro* as well as reducing the resistance of animals to infection with the test organism *in vivo*. Experiments were designed to determine whether there was any direct correlation between the *in vitro* and *in vivo* phenomena with other compounds as suggested by the activity of gastric mucin in the test tube against serum and other body fluids and in the animal against the total body defenses. Table 5 lists the substances tested and the resulting data of both *in vitro* and *in vivo* test giving their activity in inhibiting the antibacterial activity of normal serum and their ability to reduce the defenses of mice for *S. dysenteriae* infections.

The *in vitro* tests were performed by adding viable cells in the suspending medium under test to an equal volume of normal serum with a final concentration of 10^5 organisms per ml. The tubes or small flasks were then incubated at 37C and samples withdrawn over a 24 hour period for plating on agar to determine the relative increase or decrease in viable cells. The substances tested (Table 5) were made up in original concentrations twice that of the final test concentration. The highest final concentrations were as follows: mucin 2.5%, kelgin, pectin and gums 5%, starch, dextrin and gelatine 10% and dextrose at 25%. The limiting factors for some was solubility or their high viscosity made pipetting and mixing difficult. The data shown in column one of the results column in Table 5 suggest that some of the viscous substances are able to prevent the antibacterial action of normal serum from destroying *S. dysenteriae* cells in the test

tube. None of the active substances tested were quite as efficient as mucin in concentration or as easy to work with because of high viscosity. However, kelgin and pectin especially and the gums showed some inhibiting activity. The starch, dextrose, dextrin and gelatine showed no activity as tested.

TABLE 5

Inhibition of Serum Bactericidal Activity by Various Substances

Material	Inhibiting activity	
	In vitro	In vivo
Mucin	+	+
Kelgin	+	+
Pectin	+	+
Gum Tragacanth	+	+
Gum Dammar	±	±
Gum Acacia	±	±
Starch	—	—
Dextrin	—	—
Dextrose	—	—
Gelatin	—	—

+ = inhibits bactericidal action
± = slight inhibition
— = no inhibition

The same substances were investigated for ability to reduce the resistance of mice to infections with *S. dysenteriae* as listed in the second column of results in Table 5. The organisms were diluted from a young culture (18 hr.) through broth in ten-fold serial dilutions from 10^{-1} through 10^{-9}. Five ml. were added to equal volumes of the concentrated preparations of materials to be tested, mixed and six mice were injected with 1 ml. amounts from each dilution preparation. The mice were observed for deaths over a 14 day period. At death, mice were autopsied and cultured for *S. dysenteriae*. Control mice were injected with the substances diluted with broth alone for toxicity. None was noted. The findings revealed that the starch, dextrin, dextrose and gelatin were without activity and did not increase the infectivity of the organism. On the other hand, those substances showing some ability to inhibit or neutralize the antibacterial action of normal serum did have some activity *in vivo*. None of these substances kelgin, pectin and the gums were able to increase the infectivity level more than 10^{2}–10^{3} fold while the gastric mucin was able to decrease the number of organisms necessary for infection by

10^7 fold. Perhaps some lots of these substances might vary in activity as did certain lots of gastric mucin as shown previously.

Attempts to quantitate the *in vitro* test to compare the various lots of mucin as done *in vivo* in Figure 1 to show quantitative differences did not meet with great success. With the 10^5 cell inoculum, all mucin samples were able to destroy the cells within 12 hours. Similar experiments to compare *in vitro* activity of kelgin, pectin and the various gums quantitatively with *in vivo* activity was not productive. These *in vitro* tests were made by diluting the mucin or other test substances in normal serum and adding the standard number of *S. dysenteriae* cells (10^5 per ml.) to the mixture. The various lots of mucin as noted above were active *in vitro* to practically the same extent and showed only slight differences in the dilutions in which they were active in each case although *in vivo* tests showed considerable differences in activity. Another method of differentiating *in vitro* quantitative activity might be by varying the number of organisms rather than diluting the mucin. This was not done in these experiments but appears worth investigation. Tests with the other substances carried out in a similar manner as for mucin described above revealed somewhat the same result although in each case, the kelgin, pectin and gums could only be diluted 4 or 8 fold to still retain activity in neutralizing the serum while the mucin samples could be diluted 32–64 fold while still neutralizing serum antibacterial activity.

Preliminary tests were carried out with some fractions of mucin in an attempt to determine the factor or factors present in mucin which would be active *in vitro* and *in vivo*. Gastric mucin as prepared from hog stomach is a heterogeneous granular preparation which when dissolved is quite viscous and quite difficult to manipulate for fractionation. Much of the mucin is not in true solution in the 5% preparation but rather in suspension. The preliminary fractions were obtained from Dr. Norman Weisman. Since Morgan and King (1943) had found the blood group A substance to be present in gastric mucin and it was shown that this substance was able to aid *Salmonella typhosa* to infect mice, this was one of the substances tested. Others shown in Table 6 were a water insoluble residue prepared by high speed centrifugation of a mucin suspension, a water soluble portion and mucin ash. All were prepared in the concentration in which they appear in 5% mucin and used as suspending media for the

S. dysenteriae organisms for intraperitoneal injections in mice. The water insoluble residue showed considerable activity while the ash showed slight activity in comparison to whole mucin. The blood group A substance and the water soluble portion were inactive in promoting *S. dysenteriae* infections in mice. Smith and coworkers (1953) in a summary paper of an extended study on the active fractions of gastric mucin for *Salmonella typhosa* infections in mice found several active fractions. His studies using well defined chemical entities are a fine series of studies on such a difficult analytical problem presented by such a heterogeneous material as gastric mucin. Smith quite wisely suggests that these substances found active for *Salmonella typhosa* might not show the same activity for other organisms. It would be of interest to try to test the substances with *S. dysenteriae* both *in vivo* and *in vitro*.

TABLE 6

Inhibition of Serum Bactericidal Activity by Mucin "Fractions"

Fraction	Activity	
	In vitro	In vivo
Blood Group A Substance.....	—	—
Water Insoluble Residue.....	+	+
Water Soluble Portion.....	—	—
Ash	± (sl.)	± (sl.)
Whole mucin	+	+

+ = inhibits bactericidal action
± = slight inhibition
— = no inhibition

Summary. Different lots of gastric mucin have shown variable activity in increasing infectivity of *Shigella dysenteriae* for mice. Different strains of *S. dysenteriae* suspended in the same lot of mucin vary in their ability to infect mice.

Normal mouse blood, plasma, serum and peritoneal fluid have antibacterial action for *S. dysenteriae*. Heated serum shows similar activity suggesting complement mediated mechanisms are not involved. Beta lysins are proposed as the mechanism involved. Normal sera from mouse, rabbit, sheep, bovine and human origin were all found active in antibacterial action for *S. dysenteriae*.

Addition of gastric mucin to normal sera inhibited, neutralized or destroyed the antibacterial action. Attempts to characterize

the antibacterial fraction of normal plasma were not fruitful probably due to liability during fractionation or storage.

Certain gums and other high viscosity substances such as pectin and kelgin revealed slight activity similar to gastric mucin *in vivo* and *in vitro*. Other substances tested were not active in either test. Some correlation in the *in vivo* and *in vitro* phenomena are noted.

Preliminary fractionation of gastric mucin indicated the active portion was present in a water insoluble residue.

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STUDIES ON GASTRIC MUCIN

II. Spontaneous Pseudomonas Infection In Mice Following Injection of Mucin*

Frank B. Engley, Jr.

The early studies on the use of gastric mucin as a virulence enhancment or rather, a resistance lowering agent have been reviewed most adequately by Olitzki (1948). Gastric mucin has been tested as a suspending medium for activity in various experimental animals with numerous types of microbial forms. A survey of the literature has not revealed observations where spontaneous infections have occurred in experimental animals following the injection of gastric mucin alone.

It has been the standard procedure in this laboratory to test each batch of 5% gastric mucin after sterilization by culturing for sterility and for animal toxicity before use. Cultures were made by adding 1 ml. of the mucin preparation to broth and a loopful streaked on an agar plate surface. Toxicity for animals was tested by the injection of 1 ml. aliquots of the mucin intraperitoneally into six white mice (20 gram) with observations made over a 24 hour period before use of the mucin. The mice were kept for observation over a 14 day period before discarding. On occasion one of the mice might die from injection injury within several hours or from non-specific cause during the observation period. Autopsy and culture revealed negative findings or rarely a Proteus culture if the mouse had been dead for several hours before the cultures were made.

During the course of a long series of experiments in mice with gastric mucin (type 1701 W of Wilson & Co.), the control mice in one particular control test did not follow the usual pattern but all six of the control mice were dead within 18 hours following injection of the mucin. The routine cultures of mucin were negative but cultures from the heart blood of all six mice yielded pure cultures of *Pseudomonas aeruginosa*. Repeat cultures on the mu-

* From the Department of Bacteriology and Parasitology, The University of Texas Medical Branch, Galveston. Studies conducted at Biological Laboratories, Chemical Corps, Frederick, Maryland. Received for publication November 28, 1953.

cin were negative while another group of six mice from the same stock lot injected with the mucin also died within 18 hours after injection. Again *Pseudomonas aeruginosa* was isolated from the heart blood of all mice. The mice used in all studies were obtained as stock in groups of 100–400 from a central animal farm.

Since these findings did not fit into the usual pattern of tests with the mucin control mice, further investigations of the occurrence were carried out.

Cultures had ruled out the possibility of infection coming from the mucin and the remaining question to be answered then was what was the source of infection. Possible sources were the syringes, the environment of the mice, the fur and skin of the mice, or the organism might be present as a potential pathogen within the body or as a latent infection.

Cultures were taken from the syringes and needles and found to be negative. Cultures were also made from the water, food, bedding and fecal droppings of the mice. All revealed the presence of Pseudomonas organisms. Other cultures of the stock mice showed the organisms present in the voided urine, feces and on the fur of the mice but not in the heart blood.

The cultural data did not clarify the picture completely. Pseudomonas organisms were found throughout the environment of the mice and on the fur of the mice in the area where injections were made. The infecting agent could have been introduced from the outside environment by the injection procedure. The organisms were not found in the heart blood so that a bacteremia was ruled out. Further cultures of peritoneal fluid, liver, spleen, lung and kidney of the stock mice were negative. Pseudomonas organisms were found in the intestinal contents, however, as might be expected.

Stock mice were injected with sterile gastric mucin intraperitoneally after swabbing the abdominal area with 70% alcohol in the usual procedure and then transferred to cages which had been sterilized. The mice were provided with food, water and bedding which had been sterilized. Again the mice died with Pseudomonas being isolated from the heart blood as well as lung, liver, spleen and kidney.

The *Pseudomonas aeruginosa* was placed among the stock cultures and the remaining mice were discarded. All of the cages and equipment were sterilized and the animal room was disin-

fected. A new lot of stock mice was obtained from the animal farm. Injection of these mice with the same preparation of mucin did not result in deaths. Cultures of the heart blood and organs were negative at 24 hours following mucin injections.

A group of the mice from the new stock was injected with the Pseudomonas in varying doses in saline. Large numbers ($10^6 - 10^7$) were necessary to kill the mice. When injected with mucin as a suspending medium small numbers ($10^1 - 10^2$) would kill the mice. When the culture was swabbed on the abdominal surface and injections of sterile mucin were made intraperitoneally through this area no deaths occurred.

From these observations it was concluded that one particular group of stock mice harbored the *Pseudomonas aeruginosa* and that the injection of mucin so lowered the resistance that the organism was able to multiply, cause infection and death. No similar occurrence has been observed when working with numerous lots of mucin in various stocks of mice over an extended period.

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COMPLICATIONS IN THE POST-ANAESTHETIC PERIOD OF PARTURITION FROM THE PEDIATRICIANS' VIEWPOINT*

ARILD E. HANSEN

The physician who cares for children must be interested in the problems of anesthesia. If we are concerned about the anesthetic given at the time of birth, two complications are important. The first is the matter of death during early infancy. The other concerns those unfortunates who lead a pitiful and useless existence because of mental retardation, uncontrolled convulsive disorder, cerebral palsy or even a combination of these, possibly due to some complication at the time of delivery.

Deaths in infancy and childhood now are much fewer than in previous generations. Everyone is aware that life expectancy for each of us is much greater than it was for our fathers, grandfathers and great-grandfathers. This is due primarily to a decrease in deaths during infancy and childhood. In this regard it may be of some interest to consider world populations. Rather striking data have been presented by Helmholtz (1952). For thousands of years the population of the world changed very little. In contrast, during the last century and a half the increase has been tremendous. The rate of increase of the population in the years B. C. is estimated at 0.05 per cent; from 1650 to 1750, 0.29 per cent; and from 1900 to 1950, 0.75 per cent. It is now said to be in the neighborhood of 1 per cent.

There is no doubt that numerous factors have been responsible for the great change. However, none of us need stretch our imagination very much to realize that some of the improvement has been due to fewer deaths from typhoid fever, tuberculosis, and the contagious diseases of childhood such as measles, scarlet fever, whooping cough and diphtheria. Perhaps the most spectacular decrease has resulted from the lowering of the death rates during the first two years of life from diarrhea, enteritis and dysentery.

With improvement in sanitation, better understanding of nutrition and the ability to treat disease conditions, the infant mor-

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tality rate has been decreasing. However, when one further analyzes the infant death rate, we find that 60 per cent of those who succumb during the first year of life, do so within the first month of life. Of those who die within the first month of life, 83 per cent die within the first week, one-half of which deaths occur in the first 24 hours. This means that if we are going to effect improvement in the death rates of our infants, we have to look to the very early hours and days of life as being especially important.

In this connection I have drawn upon data presented by Bundeson, Potter, Fishbein, Bauer and Plotzke (1952). Of 873,310 live births in Chicago from 1936 to 1949 there were 18,741 deaths in infants within the first month of life. Satisfactory postmortem studies were made on 10,021 infants and accurate clinical investigations had been made on 8,905 infants. Ninety per cent of the deaths within the first month of life were due to the following causes:

Abnormal pulmonary ventilation—43.7%. Most of these deaths occurred in the first day, the death rate being higher in premature infants than in full term infants. In prematures the death rates were the highest following delivery by Cesarean section. This type of delivery is particularly dangerous for premature infants. The fundamental cause of this peculiar pulmonary condition is obscure.

Injuries at birth—16.6%. Again, the death rates were highest in premature infants, being high in the offspring of both the younger and the older mothers, and following the use of high forcepts delivery, version and extraction. Deaths within the first day were eight times higher than during the 15 ± 29 day period. Death rates were higher in white babies than in non-white. Incidentally, in post mature infants (over 4250 grams) the leading cause of death was injury.

Malformations—15.8%. Certain ones may be corrected surgically.

Infections—13.4%. Pneumonia was by far the most common of the infections. Deaths were 3 times higher in the 15–29 day period than on the first day. Antibiotics are recommended with prolonged labor and also in premature rupture of the membranes.

Blood dyscrasia—5.4%. Erythroblastosis fetalis (2.8%) occurred three times more frequently in the whites, and was found

mostly in full term babies. Early exchange transfusions are important in this condition. Hemorrhagic diseases of new born (2.4%) was found more commonly in prematures than in full term babies.

Anoxia—3.8%. Most of these deaths occurred within the first 24 hours. The highest rate was in the Cesarean section babies, and was higher in white than in non-white babies.

Miscellaneous—1.4%. These included endocrine, 0.2%; intestinal obstruction, 0.3%; liver disease, 0.2%; trauma (not birth), 0.3%; others, 0.3%.

Early deaths are apt to be due to abnormal pulmonary ventilation, injury, anoxia and erythroblastosis; later deaths to infection. Some conclusions drawn by these observers regarding ways to reducing deaths in infants are: 1. prevent prematurity; 2. good care after birth, as for example, clear air passages, and the proper use of oxygen, heat and humidity.

Concerning anesthetics and analgesics, data presented by these same workers may be of some significance. Data on 8,642 deaths in relation to anesthesia from Bundeson *et al.* are as follows:

Type of anesthesia	Causes of Death in Relation to Percent of Deaths				
	Infection	Injury	Anoxia	Abn.Pul.Vent.	All
None	37.8	31.6	28.3	40.4	34.5
Inhalation	52.9	60.4	64.1	50.8	57.2
Local, spinal, caudal	6.5	5.1	5.2	5.4	5.7
Analgesia, barbiturates, morphine, scopolamine	2.8	2.9	2.4	3.4	2.6
	100.0	100.0	100.0	100.0	100.0

From these data it appears that inhalation anesthesia contributes to deaths from injury and anoxia, whereas local anesthesia does not. It must be remembered that anesthetics are used in operative deliveries far more than in non-operative.

A study was made of the anesthetics administered to mothers in the year 1938 in 28,217 births. Although the data are small, the authors feel there had been little change in the policy of anesthetic administration and that these would be representative of the period. On the basis of the information regarding anesthetics given at the time of delivery in 1938 in Chicago, Bundeson *et al.* calculated figures for 873,310 infants born in Chicago from 1936 to 1949. For the 14 year period the neonatal mortality rate was

21.5, whereas with no anesthetics it was estimated to be 17.3, and with an anesthetic, 24.4.

Data regarding anesthetics in relation to the proportion of live births in Chicago for the year 1938, and on the basis of these the estimated mortality rates for 1936–1949, were as follows:

	1938 Data		1936–1949 — Estimated data		
	Number	Per cent	Live births	Deaths	Mortality rate
Total	48,217	100.00	873,310	18,741	21.46
No anesthesia	20,049	41.59	363,209	6,281	17.29
Local & regional	2,065	4.28	37,378	979	26.19
Barbiturate	136	0.68	2,445	76	31.08
Opiate	275	0.57	4,978	284	57.05
Ether	13,389	27.77	242,518	4,733	19.52
Ethylene & NO	6,217	12.89	112,570	3,792	33.69
Nitrous Oxide	2,111	4.38	38,251	1,176	30.74
Cyclopropane	239	0.50	4,367	661	151.36
Choloroform	180	0.37	3,231	27	8.36
Others & Unknown	3,356	7.37	64,363	732	11.37

On the basis of the data of Bundeson *et al.*, it appears that anesthetics tend to increase the hazard to the life of the infant. It was pointed out that mid and high forceps delivery, Cesarean section, breech extraction and version and extraction are increased hazards for the infant. It is in the management of these procedures that the anesthetist plays a particularly important role. For the sake of the infant maximum oxygenation and minimum sedation must be assured.

So far we have been speaking of the waste products of pregnancy, namely neonatal deaths, particularly in reference to those which are preventible. Even more tragic, perhaps are those unfortunate humans who are doomed to an existence of suffering and extreme limitations because of impairment of physical agility, mental function, or periodic convulsive seizures. Any or all of these may occur in the cerebral palsied child. There is no selection into whose family they come.

It has been my lot to serve as consultant at the Moody State School for Cerebral Palsied Children. Recently my colleague, Robertine St. James, P. F., Director of the institution, reviewed the neonatal data on the charts of 104 children who have been admitted for treatment. It was found in the histories that in only 10 instances did the parents state that delivery was without abnormality. The incidence of prematurity was twice that expected.

There was a history of neonatal anoxia in 13. Cesarean sections had been performed in 4 instances. Precipitate delivery was present in 14. Delivery was prolonged or breech in 25 cases. One infant was post mature. Only in two instances was the cause attributed to post natal influence—encephalitis in one, and trauma in the other. The history of juandice was frequent.

One may assume that information of this type gives a clue in regard to prevention, although it is readily admitted that only in part is the problem related to anesthesia. Each time an infant is born, some little slip in the chain of those partaking in the delivery of the child may be the precipitating factor in throwing into the world an inadequately equipped human being. The newborn needs oxygen, and the anesthetist may be the one who can give him this (through his mother). Every cog in the wheel is important in making that newborn a bouncing, pink, bawling infant, rather than the cyanotic, limp infant who some day may be spastic, convulsive or idiotic.

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THE MEASUREMENT OF PSYCHOLOGICAL FACTORS IN MARITAL MALADJUSTMENT*

M. R. HARROWER

Although psychodiagnostic examinations are a well established procedure as an adjunct to psychotherapy when a battery of tests are utilized to evaluate the intellectual and personality characteristics of a patient, little has been reported on such personality appraisals of two or more persons involved in some interpersonal difficulty and tested at approximately the same time. Several years ago, a study was reported by me, in which four members of a family, the parents and two sisters, were all tested with a view to throwing light on the problem of the elder girl, the "patient." In this, the interrelatedness of the problems of the two sisters became apparent against the background of the parents' personalities. More recent work about to be reported by Peck, Harrower, and Margolin will show the interrelatedness of the personality characteristics in members of "gangs" of juvenile delinquents who have appeared in the Children's Court in Manhattan as first offenders. These studies have thrown light not only on each individual child, but on the role within the gang which his personality prescribes that he should play.

Findings in both these investigations impressed me with the unsatisfactoriness in cases involving close interpersonal relations, of psychological examinations on one individual alone. Joint examinations would seem to be indicated, particularly in those cases of marital difficulties where, for some reason, one member of the pair has become the "patient" while the other member remains outside the therapeutic and evaluative situation. Studies on poth partners in such circumstances might give helpful information.

Methods. This report deals with 80 persons, 40 couples, who had been referred for psychodiagnostic examination by a psychiatrist or physician because their presenting problems involved marital difficulties. These 40 couples received a full psychodiagnostic work-up and in addition to the personality appraisal of each individual separately, a comparison of findings was made

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in terms of the respective depth or seriousness of the disturbance as reflected in the test findings. Sometimes suggestions as to the type of difficulties which might be expected from two persons with these respective test profiles were formulated. Occasionally prognostic expectations were hazarded. These, however, pending experimental evidence, had to be very tentative.

This investigation was in progress over a period of eight years. During these eight years, the tests which were administered were augmented. This was inevitable since new and valuable tests become available ever so often and the psychodiagnostician is, or should be, ever alert to the possibilities of obtaining more relevant material from his examinations.

Most of the 40 couples received the following six tests during the examination: The Verbal Wechsler-Bellevue Intelligence Test, the Szondi test, the Rorschach test, the Figure-drawing test, the Most Unpleasant Concept test, the Holsopple-Miale Sentence Completion Test. All couples received at least four tests, so that every personality appraised, and every comparison between the members of a given couple, was the result of material from various test instruments. In every case the same tests were given to both partners. Thus material from couples tested some eight years ago, while based on 4 rather than 6 tests, was always uniform.

The material from the referring psychiatrist relating to the couples' background, difficulties, psychiatric status, and final recommendations for therapy, ran the whole gamut from complete case histories through letters highlighting the essentials, to brief comments, to no information at all. It is clear that such material as this, which accumulates and grows without being initially part of planned research, must be subject to the hazards of the inevitable variations in individual procedures. Regardless of the extent which the research psychologists may wish to correlate findings in each case and at every stage with a psychiatric opinion, the fact remains that in clinical practice this is not always possible.

When the final assessing of these 80 cases began, a short questionnaire was sent to each of the physicians responsible for referring these patients. The questionnaire dealt with the following items: (1) Are these persons still married? (2) Did each or both go into therapy? (3) If so, for how long? (4) Did this therapy materially alter, modify or have some influence on the marital situation?

Replies from the majority of the referring physicians and account for 34 of the 40 couples. The results are epitomized as follows:

Still married	Status not known	Divorced	One partner died
18	10	5	1

The figures in regard to the effectiveness of therapy are as follows:

Therapy materially altered status of marriage	Therapy modified status of marriage	No therapy given or no effect	Died	Not estimated
14	8	7	1	4

For two couples who were counselled by me, a detailed follow-up over a period of several years was available, for ten others sufficient information was obtained so that the test findings could be verified.

Results. The psychological material was originally assessed in the usual manner, and then was re-assessed in the light of the marital maladjustments existing. A general overall appraisal from all the six tests resulted in a statement about each couple which fell into one of the following eight categories: (1) Both partners appear psychotic; (2) The husband appears psychotic, or borderline, the wife less disturbed; (3) The wife appears psychotic, or borderline, the husband less disturbed; (4) The husband appears more seriously disturbed, but not psychotic; (5) The wife appears more seriously disturbed, but not psychotic; (6) Both appear equally, and seriously disturbed, but not psychotic; (7) Both appear equally somewhat disturbed; (8) Both appear to fall within normal limits for the total population.

This classification was made twice over. In some cases, there was an interval of as much as eight years between the original and the second assessment. This is important in that it constitutes a check or validation on the procedures and on the examiner. The second assessment was made from the raw material or the raw scores and again a judgment in terms of the respective seriousness of the partners' difficulties was given. It is interesting to find that in only one of the 80 cases was the second judgment different from the first. On the more recent assessment in this one case both partners were considered to be psychotic whereas previously only one had been listed as psychotic, the other as "seriously disturbed."

These findings may be classified as follows:

Both partners psychotic	Man psychotic or borderline; woman less disturbed	Woman psychotic or borderline; man less disturbed	Man more disturbed, no psychosis
3	10	1	12
Woman more disturbed, no psychosis	**Both seriously disturbed**	**Both somewhat disturbed**	**Within normal limits, both**
5	5	2	2

Somewhat startling is the finding of the relatively large number of cases where the husband is either psychotic, (10 cases) or the more seriously disturbed (12 cases). These two groups together account for 22 husbands or over half of the total examined.

In contrast, those cases where both persons are equally disturbed, although there are 4 categories from which to draw, amount to only 12 couples; and those cases where the wife is more seriously disturbed, come to only 6 cases.

Associated with the finding that in the majority of cases the husband is more disturbed, is another item of interest. On several cases, several years apart, the examiner's notes carry a comment that despite the fact that the wife appeared to be the more seriously disturbed during the interview, her test findings seemed to belie this. On consulting several of the therapists who had referred such couples, there appears to be evidence which would support the hypothesis that the *least disturbed* partner comes to therapy first. This partner comes as the "patient" often under pressure from what turns out later, to be the more disturbed partner.

One therapist, for instance, wrote "in the cases which I referred to you certainly the healthier person went into therapy first . . . . and in another case which I have not referred but have recently seen the so-called 'crazy' wife has needed only short-term therapy while the husband is much more seriously disturbed."

Because these cases were examined at irregular intervals, and because there was no awareness on the examiner's part at the time of the "group as a whole," this trend was not apparent. However, during the initial interviews, it was clear that persons who appear equally disturbed or confused over their problems in the clinical setting made a very different impression one from the other when their test findings were assessed. It has not been unusual to find that the more distressed person at the testing interview turns out to be less disturbed in the tests themselves.

In order to focus attention on this, and to make possible a quick contrast of the performance of psychologically related persons, a summary sheet (Harrower, 1952) was developed which gives some of the highlights from the various tests. One of these sheets (Figure 1) shows how these overall appraisals can be compared at a glance. It also demonstrates that identical behavior in the testing situation does not mean comparable test performance. In this instance, one partner emerges on the test as an individual with superior potentialities, the other is shown as more seriously disturbed and inhibited.

This overall appraisal from the total test findings is, however, only a small part of what can be gleaned from this accumulation of test material. Each of the six tests used yields details which have been fully evaluated. In this paper, only the details from the Verbal Wechsler-Bellevue are being presented.

FIGURE 1

SAMPLE CHART GIVING SUMMARY OF TEST FINDINGS

HUSBAND WIFE

MANNER DURING TEST				
OVERTLY DISTRESSED	UNEASY	RELAXED, INTERESTED	COMPETITIVE AND TENSE	HOSTILE
RATE OF PERFORMANCE				
VERY SLOW	SLOW	AVERAGE	SPEEDY	EXCESSIVELY SPEEDY
I. Q. (BELLEVUE-WECHSLER)				
BELOW AVERAGE	AVERAGE	HIGH AVERAGE	SUPERIOR	VERY SUPERIOR
PRODUCTIVITY (RORSCHACH)				
MEAGRE	AVERAGE	RICH AND WELL ORDERED	RICH, BUT CHAOTIC	CHAOTIC
RELATION TO REALITY (RORSCHACH, BELLEVUE WECHSLER, DRAWINGS)				
FANATICAL EXACTITUDE	NOT NOTICEABLY DISTURBED	FIRM AND GOOD	"ARTISTIC LEEWAY"	LOOSE
USUAL - UNUSUAL THOUGHT CONTENT (RORSCHACH, UNPLEASANT CONCEPT)				
STEREOTYPED	AVERAGE	ORIGINAL	BIZARRE QUALITIES OFFSET BY OTHER FEATURES	BIZARRE
EMOTIONAL TONE (RORSCHACH, SZONDI)				
LACKING, REPRESSED	STRUGGLING FOR EXPRESSION	WARM, READILY AVAILABLE	GETTING OUT OF HAND	EMOTIONS RAMPANT
CONSTRUCTIVE FANTASY (RORSCHACH)				
ABSENT	AVERAGE	ACTIVE	VERY ACTIVE	TOPHEAVY, WITHDRAWAL
PASSIVITY - AGGRESSION (RORSCHACH, SZONDI, UNPLEASANT CONCEPT)				
HAMPERING PASSIVITY	INSUFFICIENT DRIVE	SUFFICIENT DRIVE	AGGRESSION	OVERPOWERING AGGRESSION
ANXIETY				
MINIMAL	MILD	MODERATE	EXCESSIVE	PANIC

This well-known intelligence test is composed of several subtests revealing specific abilities or lack of them, such as the in-

dividual's fund of informational knowledge, his comprehension, reasoning, and judgment, his memory for digits, his arithmetical ability, his capacity for abstractions or generalized thinking. Since the performance scales was not used, the "verbal intelligence quotient" (IQ) results from totalling the scores on all these sub-tests. But it is very important to remember, since it is so frequently forgotten, that one and the same I.Q. can be obtained from very different sub-test scores.

Figure 2 illustrates the method of scoring the Wechsler-Bellevue test, and may serve as an orientation for the non-psychologi-

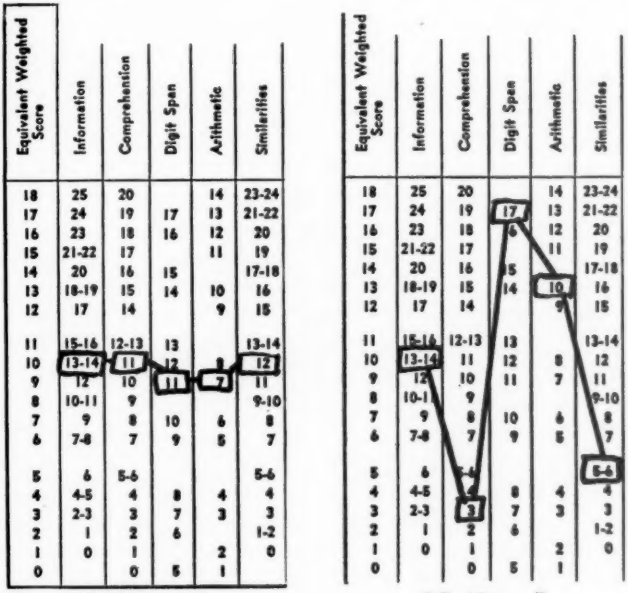

FIGURE 2

cally trained reader to the "scattergram." Both "A" and "B" have I.Q.'s of 101. Nothing could be more "average" or "ordinary" than such an I.Q. of 101 which is just on the mid-line and represents average intelligence. Yet these two individuals could not have more varying psychological equipment or have been more

different in their behavior. "A", for instance, is a uniformally-functioning, stable mediocre individual; "B", is a psychotic girl. Although both these scores add up to 101, they add up to it by virtue of very different numbers, representing very different abilities. The apparent equality of their "intelligence quotient" (IQ) is arbitrary, and may even be misleading.

Let us imagine that these two people, "A" and "B", are husband and wife and visualize these two graphs superimposed. With these respective psychological equipments, certain flagrant difficulties would be bound to exist. "B", who is now the hypothetical wife, has extremely low scores on the test for comprehension and the test for abstract thinking. She would not understand what would be relevant action nor would she be able to think logically. On the other hand, she has an excellent rote memory and is smarter at figures than "A". Although "A" and "B" both have the same score on the information test, it would be found, if the actual records were studied, that "A" loses points because he cannot define some of the more difficult or technical words (questions at the harder end of the scale), while "B" loses points because of failure to know the number of weeks in a year or the date of Washington's birthday, but excels in the more erudite information at the harder end of the scale. While this pair is hypothetical as a pair, they are actual persons who have been examined. Comparison of their Wechsler-Bellevue "scattergrams" illustrates a method by which some psychological factors in marital partners may be assessed.

As a result of such comparisons in the present study, six specific "patterns of discordancy" emerged; six variations, that is, between the "scattergrams" of the two partners. It was moreover possible to place each of the 40 couples under the general heading of one or other of these six patterns. The specificity of these patterns allowed in some cases, of a kind of checkup within the therapeutic situation directly related to the kind of difficulty reflected in the tests. For instance in one case, the wife who was the patient, repeatedly spoke of difficulties which related to her lack of ability in some areas, her "dumbness" and in contrast to her husband's exceptional abilities in these same areas. Not until the husband himself was tested, did the full implications of these difficulties both in their realistic and neurotic aspects, become clear.

Figure 3 illustrates one "pattern of discordancy." It was obtained from the husband and wife just mentioned. In this and in all other scattergrams, the black dots relate to the husband's scores while the outlined figures refer to the wife's. This particular "scattergram" illustrates several points. First we notice the husband's unusually high, uniform performance. Here is an individual whose score is only a few points below the maximum obtainable. On three of the subtests he does achieve the maximum scores. Both within our series and even in the population at large, such a uniform high level performance is very unusual. This individual (the only one in the series) was not a patient and offered to be tested in the hope of throwing light on his wife's difficulties after she had been in therapy for some time. In contrast to this, the wife's score is both much lower in general level (she would fall into the Average, rather than the Very Superior Group of the total population) and it also appears that her performance is much more erratic. While she does well on the abstract thinking, she does extremely poorly in the arithmetic.

It might be well at this point to explore a little more fully what high or low scores in the respective subtests may mean. Although we cannot go into this in any great detail, it has been reliably demonstrated that extremely low scores on "arithmetic" are almost invariably due to anxiety which sometimes reaches panic-like proportions in this subtest, rendering the individual completely incapable of functioning. A sharp drop in the "arithmetic" scores, therefore, is almost always an index that the individual may be at the mercy of disrupting and disorganizing anxiety. Particularly as in this case, when an individual is capable of logical thinking (note the much higher score on the "similarities") one may assume that one can contrast the capacity of the individual when not anxious with performance under the stress of panic.

While a drop in the score in the "arithmetic" is, let us say, a deviation from a better level and is, therefore, a "hopeful" sign, a sharp drop in the "similarities" usually indicates a more serious type of disturbance in which anxiety does not play a prominent role. For, when the score in the "similarities" is very low, it indicates that the individual is showing a distortion in thinking, that there is a disturbance in his relation to reality. Metaphorically, then, one might say the framework within which he envisages things has itself become warped and distorted.

A lower score in the "information" may mean, as we mentioned earlier, that the ordinary things in life no longer are of much interest, because the individual has withdrawn into a world of his own. This is shown by failure to know or register easy facts, as opposed to lack of educational opportunities or lack of intellectual curiosity. The latter would leave its mark by the lack of knowledge of more specific items of learning or culture.

Low scores on the "comprehension" usually reflect, in somewhat the same way as a lower score in the "abstract thinking," that the individual is unable to appraise or understand what is the pertinent thing to do in situations of various kinds. This again, reflects a more serious disturbance.

A low score on the "memory" may also reflect the impact of anxiety since the individual is too frightened to listen carefully enough to the numbers he should repeat, or it may reflect a lack of concentration due to the pressure of internal problems so that the mind is never "blank" enough to register and hold onto the incoming information.

Returning now to the husband and wife, whose "scattergrams" are shown in Figure 3, it is clear that to some extent their difficulties relate to their different levels of intelligence, but their difficulties also relate to the steadiness and the intellectual uniformity of the husband and the erraticness on the part of the wife. More specifically, however, we may say (since this was very clearly brought out over a period of time in the therapeutic sessions) that the wife becomes panic stricken. She accentuates her own "dumbness" in any situation where it appears to her that she is being shown up at a disadvantage, as when she feels herself as incompetent or not knowing as much as her husband. The discrepancy between their scores on the test for "information" are significant in this respect. Therapeutically, the hopeful aspects of this situation are the relative closeness in their scores on logical thinking ("similarities") and the understanding of situations ("comprehension"). In actual fact, the outcome here was a very favorable one and after several years of therapy, this wife's "scattergram" looked much less erratic and her I.Q. rose to a point where she reached the high average to superior group of the total population.

For purposes of discussion this type of joint "scattergram" will be referred to as *Panic Introduced by Feelings of Incompetency*. It is clear that it also represents different levels of functioning and extremes of "scatter" and "lack of scatter."

Figure 4 represents a husband and wife who have an identical type of scatter, who really have parallel scores, but one occurs at a lower level of intelligence than the other. In this instance, a follow-up was possible, which showed that the more intelligent and less disturbed husband went into brief psychotherapy which was considered to be unusually effective by the therapist. This couple is still married and was rated by the thera-

pist as showing that therapy "materially altered the existing marital conditions." Incidentally, the other members of this group also showed a satisfactory outcome subject to therapy in the case of the more intelligent partner. We have called this distribution *Parallel Scores at Different Levels* that this will be a "benign" pattern, subject to successful amelioration with therapeutic intervention.

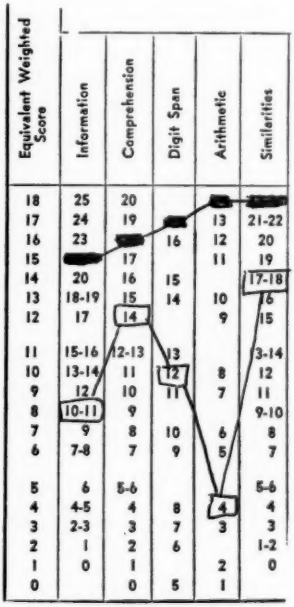

FIGURE 3

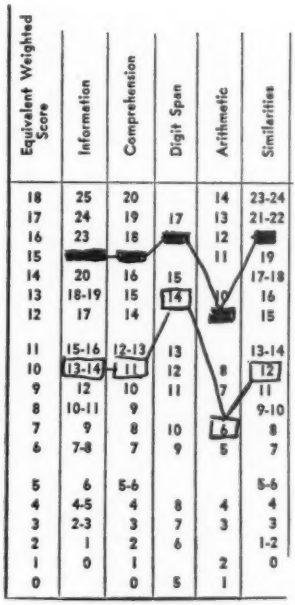

FIGURE 4

Figure 5 shows the type of interrelated "scattergram" which we have called *Cut-Throat Competition.* It will be noted that both graphs are at the extreme upper limits and that each varies only from the hypothetical ideal score by exceedingly small amounts. The basic competitiveness of these two partners presented rather fundamental difficulties which can be understood from the scores. Apparently, too great a similarity in intellectual achievements does not allow sufficient "room" for individuality to develop. Both these partners vied for the same role

in the relationship. In terms of gestalt psychology, neither partner seemed able to become the "ground" against which the "figure" could show up. Both partners needed to be the "figure" at all times. It is an interesting sidelight that each individual confessed rather shamefacedly after the examination that they had attempted to find out what tests were to be used in order that they might memorize some of the answers.

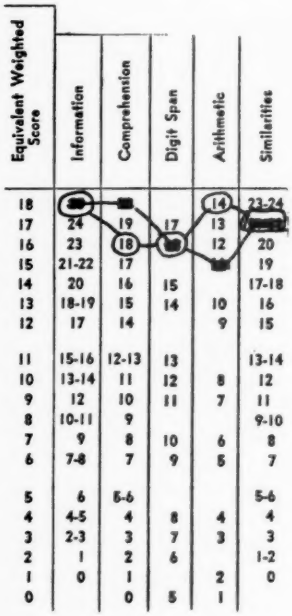

FIGURE 5

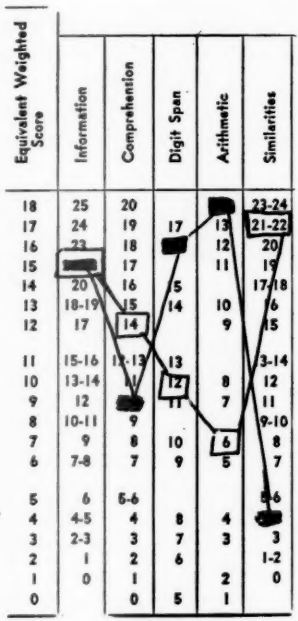

FIGURE 6

Figure 6 reflects a difficulty, perhaps the most significant of all, which involves gross distortions in thinking on the part of one partner and the panic-like reaction of the other. In this case, the husband who brought the wife to therapy, was not examined until much later, but when he was examined, an underlying schizophrenic process (reflected here in the completely atypical or distorted thinking) was brought to light. It then became evident that the wife's acute anxiety (note the low score on the

"arithmetic") related directly to this realistically frightening but insidious quality in the husband's thinking. From this and other cases, we would make the suggestion that this becomes a vicious cycle in which the panic-stricken partner becomes more and more disorganized and unable to cope with (because unaware explictly of) the distortions in thinking of the other. It should be noticed in the "scattergram" that there is a tremendous discrepancy between the scores for abstract thinking and a wide discrepancy, but in the opposite direction, in the "arithmetic." *Conceptual Distortions Producing Panic* might be suggested to describe this type of paired "scattergrams."

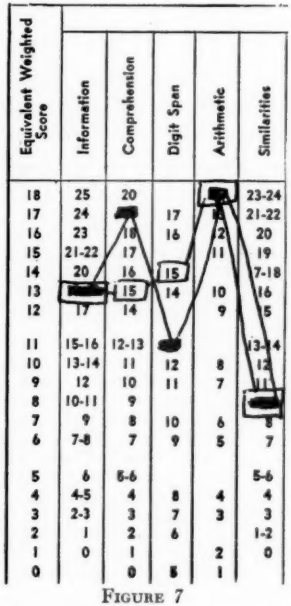

FIGURE 7

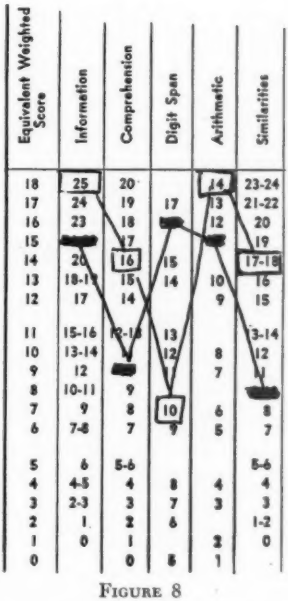

FIGURE 8

Figure 7 represents similar types of distoritions in both partners. It will be noticed that both individuals here show atypical thinking (low scores on the "similarities") and that both are outstandingly accurate in the "arithmetic," and that the rest of the graphs differ very slightly, one from the other. Interestingly enough, this does not produce panic reactions and unlike the

graphs in Figure 6, the marital adjustment of such couples appears much more amenable to therapeutic help. One couple in this group was followed over five years following brief therapy for each with the same therapist. *Similar Distortions of Thinking* might be said to describe these patterns.

The final Figure 8 reflects the chaotic distribution and wide scatter of two gifted but borderline partners. It will be noted that there is extreme erratic performance in both cases, with a variation of 10 points for one partner and 8 for the other. There is considerable discrepancy between the scores for virtually every one of the five subtests. Such couples pose very difficult therapy problems and such graphs might be described by the term *Excessive and Deviant Scatter.*

Discussion. In Table 1 is summarized a classification of marital partners disclosed by the psychological testing of 40 pairs of husbands and wives. It is significant that the psychological testing methods used are sufficiently quantitative to make their classification possible. Further, the case histories where available, indicate that these tests are fairly reliable as indices against which the results of psychotherapy may be evaluated.

TABLE 1

Types of psychological patterns observed in "scattergrams" of 40 pairs of maladjusted husbands and wives as examined by the Wechsler-Bellevue test

Type	Number of pairs	Suggested Prognosis
Panic induced by feelings of incompetency	7	Possible improvement with therapy
Parallel scores at different levels	9	Good with therapy
Cut-throat competition	5	Doubtful
Conceptual distortions producing panic	11	Doubtful
Similar distortions in thinking	4	Good with therapy
Excessive and deviant scatter	4	Doubtful

For centuries, physicians, clergymen, lawyers and thoughtful laymen have been aware of the probability that psychological factors are involved in marital discord. Vague and intuitive analyses of such factors, with lack of quantitative methods of measuring them, have been serious obstacles in the rational management of marital maladjustment. Modern methods of personality appraisal, using standardized and quantitative test-

ing methods, may furnish the badly needed foundation for the systematic development of a rational and effective way of handling marriage difficulties due to psychological factors involved in the personalities of marital partners.

Many more pairs, carefully tested, and followed for at least five years, will be necessary in order to determine the extent to which the patterns so far noted may have prognostic value in the marriage situation. The finding, in this series of 40 pairs, that more than half of the husbands are psychotic or seriously disturbed, appears to be significant statistically. Of psychological significance seems to be the observation that the more disturbed partner is usually the one who comes last for treatment.

Summary

Six "patterns" formed by the combined scattergrams of husband and wife in maladjusted couples have been shown to exist. The distribution within our series was as follows: (1) *Panic introduced by feelings of incompetency* 18 per cent; (2) *Parallel scores at different levels* 23 per cent; (3) *Cut-throat competition* 14 per cent; (4) *Conceptual distortions producing panic* 27 per cent; (5) *Similar distortions in thinking* 9 per cent, and (6) *Excessive and deviant scatter* 9 per cent. At least one example from each group has been followed carefully over a period of time so as to correlate the problems which appeared in the therapeutic interviews with the type of difficulty which would be expected from the intellectual pattern as shown on the Wechsler-Bellevue test. These patterns would appear to have value in a quick assessment of the type of difficulties which the couples are facing, and may be used within the marriage counselling situation to give objectivity to the problems in some cases. The combined patterns have also been used as a measuring rod to demonstrate changes which have occurred after therapy. More cases are needed with full follow-up, in order to estimate the prognostic significance of the classes of patterns disclosed.

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TERATOMA OF THE NECK—REPORT OF A CASE

E. C. HINDS,* W. D. SEYBOLD,† AND S. C. WALKER‡

A great variety of tumors occur in the neck. The primary ones occur (Bale, 1950) in association with organs which normally are situated in the neck and (Chapman, 1941) as a result of errors in development in an area which is a complex one embryologically. Not all of the tumors of the neck, which are the result of such errors in development, make their appearance clinically in infancy or early childhood. For example, branchial cleft cysts and thyroglossal cysts, the two most common and the best known members of this group, often do not appear before adolescence and maturity. Teratomas of the neck, however, are almost invariably evident as tumors at the time of birth or in early infancy. Their rarity accounts for the meager attention they have received in surgical literature. In a recent review of the literature, Bale (1950) was able to find 61 cases. Since that time at least 6 additional cases have been reported (McGoon, 1950; Perkins and Pautler, 1953). Errors in diagnosis and absence of records in a significant number of cases which had early fatal termination may explain in part the small number of cases reported.

Case Report. A three-year-old negro child had had a painless, slowly enlarging mass on the right side of the neck since birth. The tumor which was approximately nine centimeters in diameter lay below the angle of the mandible and anterior to the upper end of the sternomastoid muscle. The preoperative diagnosis was branchial cleft cyst. The mass was removed on January 16, 1952, through an oblique incision parallel to the flexion creases immediately over the tumor. Grossly, it was well encapsulated, lying beneath the platysma and being overlapped by the anterior edge of the sternomastoid muscle. Posteriorly, it was in contact with structures in the carotid sheath. The lower pole of the mass was adherent to the superior pole of the right lateral lobe of the thyroid. The patient's convalescence and subsequent course have been uneventful.

The tumor was nine centimeters in greatest diameter and completely surrounded by a thick fibrous capsule. There was one small cystic area with a thick yellow wall buried in gray-white, fleshy, otherwise solid tumor mass

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(Fig. 1). The bulk of the tumor was composed of poorly differentiated nervous tissue (Fig. 2). A few well defined nerve cells were present (Fig. 2). Epithelium of the skin, respiratory tract, and intestinal tract were identified (Figs. 3, 4 and 5). Thyroid gland (Fig. 6) cartilage (Fig. 7) and smooth muscle were also present within the tumor. The pathological diagnosis was teratoma of the neck.

FIG. 1. Gross appearance of cut surface. Note cystic changes.

Discussion. Nearly half of the cases reported have had surgical excision. Most of the remaining were in stillborn infants or in the newborn, dying shortly after birth. The only symptom may be the presence of a mass, but many of the tumors have produced varying degrees of respiratory obstruction, which has been the

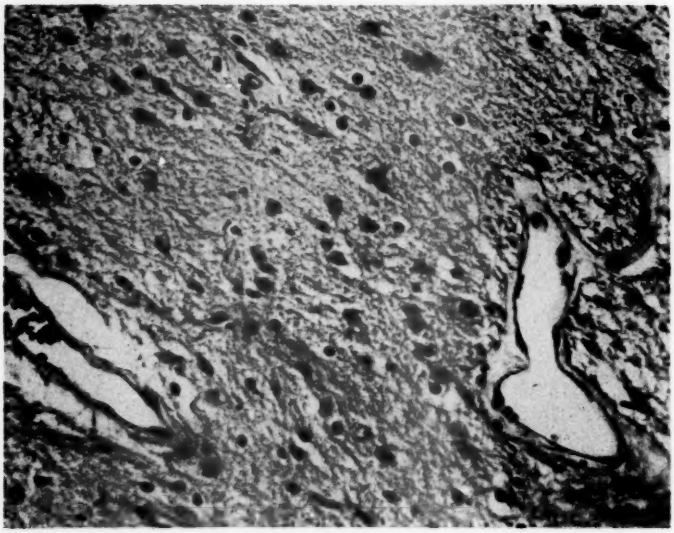

FIG. 2. Low power field showing poorly differentiated nervous tissue.

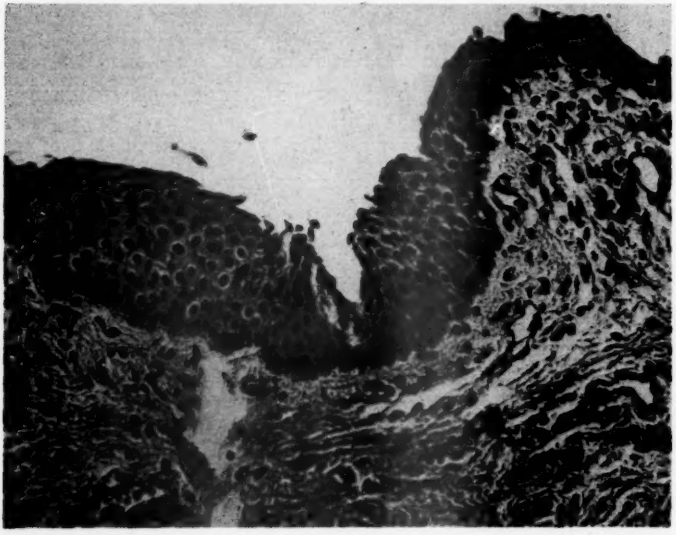

FIG. 3. Section through an area showing stratified squamous epithelium.

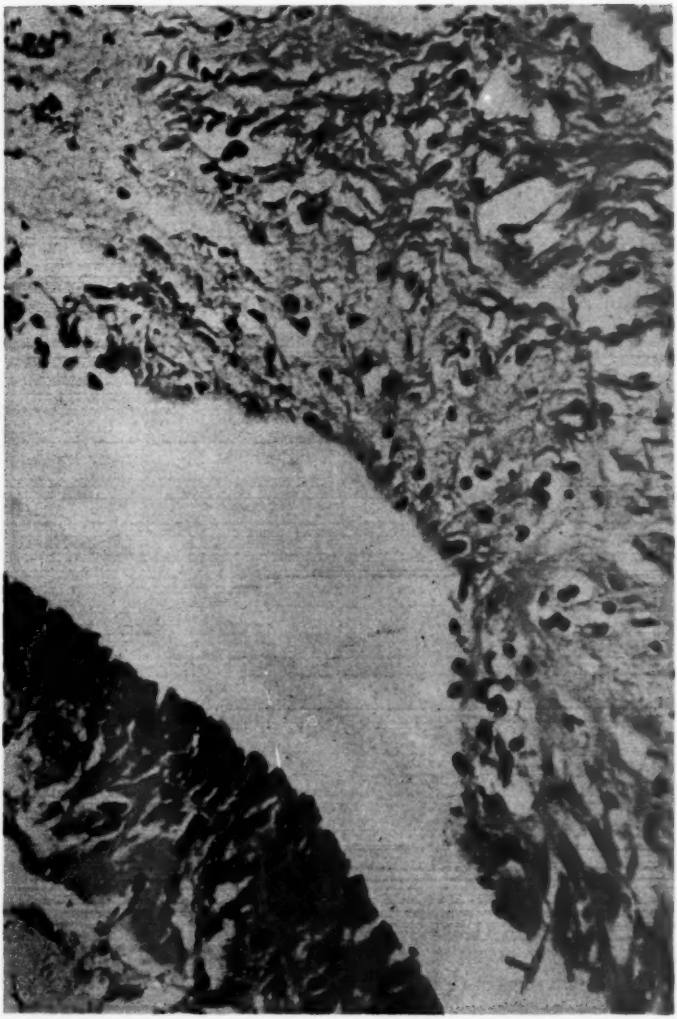

FIG. 4. High power field showing pseudostratified columnar epithelium with cilia.

leading cause of death. Bale (1950) records three of these tumors which probably had undergone malignant change. These tumors are usually enclosed in a complete fibrous capsule and can be removed easily from their bed.

FIG. 5. High power field showing an epithelial structure suggesting gastrointestinal tract.

The tumor reported here was smaller than most that have been recorded. The position and shape of the mass suggested the more common branchial cleft cyst. Many teratomas have been situated in the median plane in the region of the thyroid gland. Congenital goiter is the lesion most likely to be confused with such a lesion. A tumor such as this should be removed as soon after birth as possible. Some infants who have died probably could have been saved by early operation before respiratory complications developed.

Summary. Teratomas of the neck are apparent at birth. Many of them have caused death by tracheal compression, and in a considerable number of the cases recorded, death has occurred in the pre-natal period. However, respiratory obstruction has

FIG. 6. An area of the tumor composed of thyroid follicles containing colloid material.

FIG. 7. Area showing mature hyaline cartilage.

caused death in a number of infants born alive in whom prompt operation might have been lifesaving.

In the case here reported, the tumor produced no symptoms. It was mistaken for the more frequent branchial cleft cyst.

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REVERSIBILITY OF THE PHENOMENA INDUCED BY EXCRETORY DUCT LIGATURE IN THE RAT SUBMAXILLARY GLAND*

L. C. JUNQUEIRA AND M. RABINOVITCH

The ligature of mice and rat submaxillary gland excretory duct causes an inhibition of secretory processes, accompanied by gland atrophy and cytochemical and biochemical changes (Junqueira 1951). These results led to a study of biochemical phenomena related to cell secretion (Fernandes and Junqueira 1953). We offer here a) the histological changes observable in glands after a long period of duct ligation and b) the histological and biochemical modification promoted by duct recanalisation of previously ligated glands.

Material and Methods. Adult Wistar rats were used, weighing from 350 to 513 g. Ligature of the excretory duct of the right gland was performed as described (Junqueira 1951), leaving the left glands as controls. Nine animals were killed (nembutal overdosage) 39 days after duct ligation and their glands studied as described below (Group A). In 7 animals (Group B) the ligature was sectioned 14 days after its performance and the animals killed 21 days later. Previous experiments had shown that a 14-day period is sufficient to induce definite changes in the ligated glands (Rabinovitch *et al.* 1952). Animals were killed from periods ranging from a 7 to 390 days after ligature in order to study the effect of long periods of ligature on the histology of the gland.

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All figures are from hematoxylin-eosin stained sections, phase contrast photomicrography.

FIG. 1. Normal control gland. Observe cells of tubular portion with secretory granules in the apical region and mitochondria in the cell basis. x 600.

FIG. 2. Section of gland 390 days after excretory duct ligation. Observe absence of nuclear degenerative phenomena and maintenance of tubular structure. x 600.

FIG. 3. Control gland from an animal of group A. Observe secretory ganules in the cells of the tubular and acinar portions. x 1500.

FIG. 4. Thirty-nine-day ligated gland (group A) no secretory granules present. Absence of nuclear aspects indicating degenerative processes. x 1500.

FIG. 5. Gland of animal from group B (14 days ligated and 21 days after ligature section). Observe a return to the normal aspect with the presence of secretory granules and mitochondria. x 600.

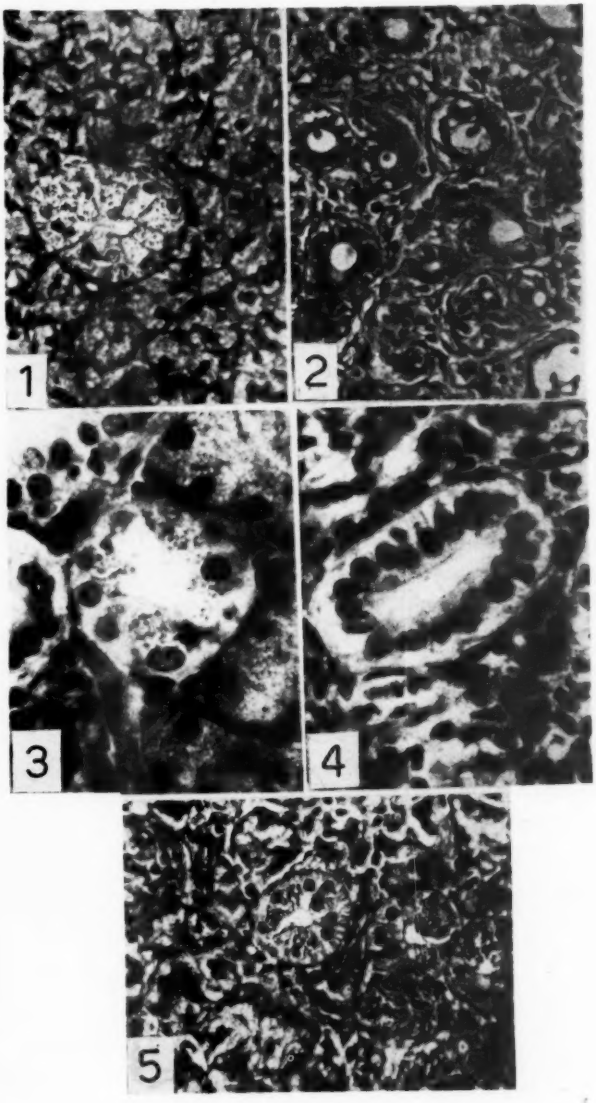

PLATE I

The histological aspect, of Helly fixed, hematoxylin eosin and Mallory stained glands was studied. Protease activity was measured at pH 8 following Anson's (1938) method. Alkaline and acid phosphatase activity determinations were performed according to the method of Greenstein (1942). Ribose and desoxyribose nucleic acid phosphorus content was estimated as described by Rabinovitch *et al.* (1952).

Results

The histological aspect of normal and duct ligated glands has already been described (Junqueira 1951). Glands of animals whose duct had been ligated from a week to a year previously showed no major changes, presenting no aspects that might suggest degenerative alterations (Figs. 1 and 2). In the glands of animals whose duct ligature had been sectioned (Group B) a definite tendency to a return to the normal histological aspect was observed.

Secretory granules reappear in the tubular and acinar portions of the gland. Mitochondria that had practically disappeared are present as rods in the basal region of the cells of the tubular portion (Figs. 3, 4 and 5).

The biochemical results are summarized in Table I. One may observe that with exception for the values of acid phosphatase the results for gland weight, protease and alkaline phos-

TABLE 1

	Number glands	Gland weight mg.	Protease activity[1]	Phosphatase activity[2] alkaline	Phosphatase activity[2] acid	RNAP[3]	DNAP[3]	RNAP DNAP ratio
		234.6	576.5	2.831	1.850	88.1	33.4	
Control	16	± 18	± 107	± 270	± 229	±12.1	± 4.3	2.64
		(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	
		150.7	395.0	2.024	1.604	52.7	42.5	
Recanalized (Group B)	7	± 113	± 170	± 509	± 257	± 17	± 8.9	1.25
		(64.2%)	(68.5%)	(71.4%)	(86.7%)	(59.8%)		
		76.4	266.0	1.757	1.869	51.2	49.6	
Ligated (Group A)	9	± 9.8	±62.8	± 247	± 249	±21.7	± 4.5	1.07
		(32.5%)	(46.1%)	(62.0%)	(101%)	(58.1%)	(148.5%)	

[1] Expressed as γ of tyrosine set free by 100 mg of tissue (wet weight).
[2] Expressed as γ of phenol set free by 100 mg of tissue (wet weight).
[3] Expressed as γ of P contained in 100 mg of tissue (wet weight).

phatase activity and nucleic acid phosphorus content are always intermediate between those found for the ligated and control glands showing that the modification induced by ligature are reversible.

The facts observed that ligated glands for long periods maintain their histological structure, and that the histological and biochemical changes induced by duct ligation are reversible, support the opinion that we are not dealing with pathological phenomena.

Summary. The ligature of the excretory duct of rat submaxillary glands leads to an atrophy of these glands and suppression of secretory phenomena. The histological aspect that appears after the first week of duct ligature remains without major changes during a period of up to one year. The section of the excretory duct ligature promotes an increase in the gland weight, its protease and alkaline phosphatase activity and ribose-desoxyribose nucleic acid phosphorus ratio.

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THE PLACE OF A PSYCHOLOGIST AMONG SCIENTISTS*

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In which field and by virtue of which methods psychology has won recognition as a science, is not the purpose of this discussion. Yet, the very fact that this question can be raised is relevant to our topic, for consciously or unconsciously, psychologists have, since the beginning of the experimental era, been influenced in the choice of their topics and the philosophy of their methodology by their desire to be accepted as scientists. Today this tendency finds its most pointed expression in "Behaviorism," that group of tenets which rejects all "subjective introspection" in favor of "objective observation" and therefore prefers animals to human subjects, because, while the latter may wish to report their own private, and therefore unscientific experiences, the former simply allow themselves to be objectively observed.

If psychology could do nothing better than follow this precept, then, however important and valuable its contributions to the total sum of our knowledge might be, it would have no characteristic that would give it a unique position in the family of sciences. It would, as a branch of the biological sciences, be closely related to physiology, and the task of delimiting the various biological sciences would have comparatively little general or philosophical interest. If this were the opinion of the author of this essay, he would not have attempted to write it. And thus the thesis which the following pages are intended to prove is that psychology has a function which no other science can fulfill, a function, moreover, which brings the psychologist near to, if not within, the confines of the philosopher's realm. This function derives from a task which the psychologist shares with no other scientist and which, at the same time makes him, in principle, though

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* This essay was written in 1940 by the late Professor Kurt Koffka (1886–1941), while spending a year as Visiting Professor at the Nuffield Institute, and the Military Hospital for Head Injuries, Oxford. It was prepared for presentation to a group of general scientists, and was to have been published in *Man and Science*. Professor Koffka's untimely death, and World War II, prevented its appearance. The current timeliness of the subject has suggested its publication now, and arrangements for this have been made by Professor Koffka's associate, Doctor M. R. Harrower of New York City.

certainly not in fact, indispensable to all scientists. The physicist has the task of explaining inorganic nature but he does not consider the working of the physicist as a part of his science, nor does the biologist treat of the biologist, even though the species of homus sapiens belongs to his field. But the psychologist cannot in any sense build a system of his science that has not a place for the psychologist, or, for the matter of that, for the scientist in general. And this simple assertion entails the unique position that will be claimed for the psychologist.

Two lines of argument lead to this assertion. In the first place psychology is the science of behavior in general and of human behavior in particular. Scientific work is human behavior, and therefore belongs ipso facto to the domain of the psychologist. Thus formulated this argument seems obvious and does not, for this very reason, reveal all the implications of its conclusion. For it might seem possible that psychology, after having developed certain explanatory principles, could simply apply them to that form of behavior that is called science. But such an interpretation overlooks an essential characteristic of scientific behavior which distinguishes it, if not in kind so in degree, from many other kinds of behavior: the results of scientific behavior, the discoveries, deductions, generalizations, are more or less permanent, and, what is even more important, they are not merely in actual fact, contingently, connected with the preceding behavior, but stand in an intrinsic, necessary, relation to it. If, therefore, one chose as the prototype of all behavior a concept that lacks this characteristic, then one could not explain scientific behavior, because one would be unable to deal with its most significant aspect. Concepts of this kind are the reflex, the conditioned response, the association, all three representing prototypes of purely contingent relations.

Let me demonstrate the truth of this statement by the example of the conditioned response. In its purest form, today hardly accepted by anybody, it establishes a purely external connection between a stimulus and a response, based on mere coincidental repetition. To repeat the classic example of Pavlov's dog: a dog when stimulated by the sight or smell of food begins to salivate, but does not do so when a bell is rung. If however, for a number of times a bell is rung each time before the dog is presented with food, then the mere ringing of the bell, unreenforced by presenta-

tion of food, will produce increased salivation, an effect which will gradually disappear when the "conditioned stimulus" of the bell has gone unreenforced a number of times. On this well established fact, and many others of the same kind, the conception of the conditioned reflex is based, a conception which in the opinion of many psychologists is a, if not the, fundamental explanatory concept of psychology. The facts, to summarize, are these: it is possible by suitable experimental procedures, to connect almost any response with any stimulus, proof that the connection thus established is purely external. It is a long cry from the statement of this fact to the theoretical position that holds such external connections as typical of *all* behavior. Another view would be that the conditioned response is the extreme case of a much more general form of connection, and that the externality of its connection is due to the very special conditions under which this behavior is elicited. But this is not the place to give a theory of the conditioned response. What we wanted to demonstrate was that scientific behavior cannot be explained by the concept of the conditioned response, because the response, being coupled to its cause, the stimulus, in an entirely external fashion, cannot be called either true or false, whereas these predicates dominate the pursuit of science. True and false imply internal relations, and such play no part in our concept.

Therefore, the fact that the psychologist has to include himself, and every other scientist, in his subject matter has far-reaching consequences for psychology itself: it determines the choice of its fundamental explanatory concepts; for these concepts must be such that they explain internal relations characteristic of the results of scientific behavior.

The other line of argument which we are to follow starts in the realm of physics. Physics is based on observation and deduction. Observation of nature requires natural events to be observed and observers who observe them. Thus every physical observation depends upon two kinds of factors, those outside and those within the observing organism. The physicist aims at establishing laws of nature that are free from the observer, and every great progress in physics up to the general theory of relativity has been a step in this direction. A history of physics written from this point of view would be highly significant, because it would reveal the different devices which the physicists have developed in

order to reduce the influence of the observer to a minimum. One of the most eminent astronomer-physicists of our time has clearly discerned the fundamental importance of our problem. Sir Arthur Eddington believes that the solution consists in eliminating more and more sense organs from physical observation, until no more than one eye is used and, since Einstein, no more than the central part of its retina, because most observations in modern physics are reduced to pointer readings, *i.e.* the perception of coincidences of a pointer and a mark on a scale (Eddington, 1935).

The principle underlying Eddington's solution is the assumption that the fewer nerve fibres of the organism are excited for a single observation, the less will this observation depend upon the organism, the more will it be determined by the natural object itself.

That this assumption is incompatible with several facts will be pointed out presently. But first we should ponder the fact that pointer readings of some sort or other have gained such importance; for clearly, the reason cannot lie in the phenomena observed by pointer readings; it must lie in the observer, and therefore this choice can find its final justification only through the study of the observer, *i.e.* through psychology.

If through the use of pointer readings the physicist expects to eliminate, or reduce to a minimum, the subjective component of observation, a theory of pointer reading as a particular form of behavior would seem desirable. Such a theory presupposes a detailed and accurate description of the act. Roughly this would run somewhat as follows: an observer who makes a pointer reading sees two larger units, a pointer and a scale, the latter being subdivided into smaller units the marks of the scale; the simple unit, the pointer, moves across the scale until it stops in the neighborhood of one of the marks. This mark then has to be distinguished from all others, has to be given a name, say 10.5, and recorded.

This cursory description shows that, considered as a piece of behavior, the reading of a pointer on a scale is by no means a particularly simple performance. As a matter of fact it is less simple than many a procedure that has been abandoned in favor of it. To test a substance with one's tongue in order to find out whether it is sweet or salty, to observe litmus paper turning red or blue, these are behavior forms a good deal less complex than that of reading a pointer.

Let us analyze its complexity: it presupposes knowledge of a value series on which each individual mark has its place; it entails the perception of motion, the existence within the visual field of separate units, and the apprehension of the relation between two or three such units—the position of the pointer with regard to one or two marks; and most generally, it requires the establishment of space and objects within it.

If of all these achievements required of the organism of the observer who wants to take a pointer reading we single out the simplest, the segregation of individual units for further analysis, we find that the formation of such units demands that the field comprises more than these units; for unit formation requires inhomogeneities in the field. And for this very reason it is impossible to do what Eddington claims to be Einstein's contribution, viz. to cut down the functioning part of the sense surface to a small minimum. Much more than that is required in order to produce space and objects within it; as a matter of fact patients with retinitis who have left as much intact retina as Eddington accords to the physicist are, for all practical purposes, blind, certainly unable to take pointer readings. Eddington is, in a way, aware of this, for he mentions that the Einsteinian limitation of the observer to a small central part of one of his eyes makes it impossible for him to recognize form or extension in the external world. But he overlooks that then the achievement which he still deems possible, viz. the apparent coincidence of two things, is also abolished. Thus the attempt to reduce the influence of the observer on his observations by reducing the number of fibres of the optic nerve breaks down at the very place where it seemed to have been demonstrated: such reduction would make pointer readings impossible.

This attempt must, however, appear absurd when one remembers what Kohler (1939) has pointed out that it is not true that all physical observation is limited to pointer readings. The physicist must know what his pointer indicates, volt or ampere or temperature or velocity or what not. In order to do that he must observe the entire set-up, the way in which his measuring instruments are connected with the system whose properties he wants to measure; all this is surely very much more than pointer readings, although like those it intails the existence of segregated units in the field and the apprehension of a definite network of rela-

tions between them. Thus the organism of the observer, which seemed to have been so nicely eliminated by the device of pointer readings, remains in the observation situation as an integral part. The physicist cannot get rid of it, and therefore, in order to evaluate its influence on observation, he needs the psychologist to study the organism.

Physics, as we said before, is not based on observation alone but implies deductive reasoning. For far from being a mere collection of fact it is a rational system in which all available facts can be logically derived from a minimum of factual relations. Such a system requires concepts and ways in which concepts are interlocked, inferences, deductions, proofs. It is obvious that concept formation, inferring, deducing, are all forms of behavior; they occur within the organism, and yet without them no knowledge of the world outside the organism is possible. And thus it seems that the whole attempt of the physicist to free his data from subjective factors is futile. The problem of an objective science dependent on subjective activities was recognized in its full scope by Kant, and his solution has influenced philosophers to the present day. The rational factors of knowledge, so it says are *a priori*, i.e. independent of experience. They express the essential forms of the mind, and scientific knowledge is developed by catching within these forms the irrational, contingent, *a posteriori* elements. Scientific knowledge thus is "objective" because within the compass of our experience it is necessary. But apart from our experience it can claim no validity whatever. Nature follows the laws of thought because only what can be grasped by our forms of intuition and reason can become nature for us. Aud thus the rationality of nature, far from being the rationality of a universe independent of us, is the rationality of our own spiritual essence. It seems strange that Eddington who tries so hard to eliminate the subjective element in observation, adopts a view very similar to this.

For our purpose it is not even necessary to show why the Kantian solution is inadequate; for a consideration of the *a priori* itself, quite apart from its connection with *a posteriori* material will confront us with our particular problem. Mathematics has a truth of its own, independent of all experience, and quite apart from its application to problems of physics. How is such mathematical truth found, how is it known to be truth? As soon as one

begins to answer these questions one has to leave the realm of timeless "subsistences" and is faced with concrete events occurring in specific places and at particular times; for discovery and recognition of truths are parts of human behavior. And therefore, quite apart from the Kantian problem, the question arises how behavior, an empirical occurrence, can lead to truth, a timeless *a priori* state of affairs. How can anyone know that proposition A is true, proposition B false, unless proposition A were, either by itself or within a larger context, different from proposition B, different, that is, in such a way that its truth stands out against the other's falseness? But this is only saying that at the bottom of the logical difference of true and false there lies again a psychological problem: what characteristics must thought processes have in order to carry the "tone" of true or false?

Thus the psychologist is needed by the physicist not only when he observes but also when he systematizes his observations. And yet this is not the whole story; for the argument bends back on its origin like a snake that eats its tail. Thought processes are impossible without simultaneous brain activity. But can we ask the same question about this brain activity, this physical event, which we have just asked about the thought process? Can we demand that the brain processes corresponding to the true thought of proposition A are in the same sense different from the brain process corresponding to the false thought of proposition B, in which the thought processes themselves are different?

Perhaps the same problem will appear less speculative when it is given a slightly different twist. Thought processes usually issue in some kind of action that involves innervation of muscles and movement of limbs. Now these latter are palpably and incontestably events in the world of the physicist, and therefore their occurrence should be explained by him. But the dilemma with which the physicist finds himself confronted when such muscular activity is the direct expression of a thought process has been most lucidly formulated by Eddington: "Suppose that I have hit on a piece of mathematical research which promises interesting results. The assurance that I most desire is that the result which I write down at the end shall be the work of a mind which respects truth and logic, not the work of a hand which respects Maxwell's equations and the conservation of energy. . . If the mathematical argument in my mind is compelled to reach

the conclusion which a deterministic system of physical law has preordained that my hands shall write down, then reasoning must be explained away as a process quite other than that which I feel it to be. But my whole respect for reasoning is based on the hypothesis that it is what I feel it to be."[1] To this statement I have only one objection: it sounds as though the physicist Eddington abandoned the solution of a problem in physics to the philosopher Eddington; and I cannot admit that a physicist has a right to delegate the solution of his problems to anybody else. If the physicist assumes that every physical event must be connected with preceding and simultaneous other physical events in such a way that if the latter are given, the former must occur,[2] while if they are absent, the former will also fail to appear, then the writing down of the solution must be in this way connected with other physical events. And then Eddington's dilemma can be expressed as a type of over determination: the writing down of the correct solution is on the one hand completely determined by antecedent physical events—in the nervous system of the scientist—and on the other, equally completely in all essentials,[3] by his preceding thoughts.

But philosophically we cannot tolerate such over-determination. We should be apt to abandon either determination for the other, and thus either accept physical determination and then consider the mental and logical determination as a purely illusory epiphenomenon, or believe in the power of the mind to discover truth and then abandon the previously formulated fundamental axiom of the physicist and instead admit cases in which events outside of and different from the realm of physical nature break into this realm and impose upon events that occur within it laws that are alien to them.

Eddington sees very clearly that the first alternative is impossible; and so he accepts the second. But, as I said before, that seems equally inadmissible to me.

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[1] c.p. 90–91.

[2] It makes no difference for this argument whether one substitutes for the word "must" the expression "is very probable to occur"; for the probability of such global actions is always as great as to be practically indistinguishable from certainty.

[3] There are aspects of the transcription that are not so determined; the size of the letters, is one such; another would be whether they are written in long hand or on the typewriter.

True enough, the second alternative is not absurd, it is free from internal inconsistencies or contradictions, which the first is not. But if we accept it, we thereby relinquish once and for all the idea of a unitary system of science and put in its place an ultimate dualism, if not pluralism, of modes of being and acting, without any possibility of understanding the relationship between these different spheres. For if mind is utterly different from nature, how can it have knowledge of nature? The view under consideration can produce no answer to this question other than accepting such knowledge as a fundamental irreducible fact, for which it does not even claim to have an explanation. And in practice, what does it mean to accept two kinds of entities and laws? Where will the physicist be justified in stopping short in his tracks and ascribing the rest of the observed effects to a non-physical factor? Has any one yet demonstrated a criterion for such a decision? But as long as this has not been done, as long the physicist, so it seems to me, has no right whatever to stop anywhere; instead he has, as a physicist, the moral duty to persevere to the bitter end.

It seems that this argument leads back to our over-determination which, I said, was philosophically intolerable. But there is one assumption—and I believe only one—which makes it possible to retain both sides of the dilemma and yet avoid the blight of over-determination. This assumption is the opposite to that which made Eddington's statement so incisive, his dilemma so real, the ordinary assumption, namely, that physical events and those characteristics of mental events which have to do with truth and logical necessity are fundamentally different from each other. One can formulate the difference by saying: that nature is a realm of facts, whereas mind is a realm also of "requiredness" to use this general term coined for this purpose by Köhler (1939). But to those who like the classical physicists are not shaken by Humean skepticism and post-Humean positivism and believe in a true causal necessity in nature, the difference is one between two kinds of requiredness. Blind necessity, factual necessity as it were, in nature, intelligible necessity in mind. The corresponding categories would be cause—effect on the one side, reason—consequences on the other. Or one can say it again in Eddington's words, " 'Ought' takes us outside chemistry and physics."

The new assumption which would, as I said, do away with the over-determination of certain events, is simply that this separation of mind and matter is grossly exaggerated, that instead both fact and requiredness are denizens in either realm, and that again, both in nature and in mind, requiredness may vary as to the internality of its relation. Under this new assumption Eddington's dilemma would disappear, for the antecedents of the final act of writing down the solution, whether in the realm of thought or physical events would have the *same* character which made first the solution itself and then its recording required. According to this assumption, then, something of "ought" is inherent even in physics and chemistry.

The name of this assumption is psychophysical isomorphism. It is a hypothesis which it is the psychologist's task to confirm or refute. How this can be done, and what steps have already been taken on this road, that cannot be reported in this essay. But isomorphism as a psychological problem does indeed give an unique position to the psychologist among the scientists. For, whatever his present achievements are, he alone can ultimately give scientific justification to all science, just because science is part of his subject matter.

We shall summarize the different aspects of his contribution which we discussed in the course of our argument by repeating and developing the main points.

All science is based on observation, and observation is dependent upon the observer. The attempt to minimize the subjective element of experience by reducing the functional sense surface of the observer and therewith the type of observed data has been shown to be a failure. All observation remains subjective in the sense that it depends upon the organism of the observer, and no trick employed by a physicist can modify this fact. It loses its menace for the objectivity of observation if one treats the study of the subjective observation as a science no less objective than physics. For the observation of any physical event, the motion of a pointer across a scale, or whatnot, is as much a natural event as that physical event itself. It takes place in an organism whereas the physical event takes place outside an organism, *e.g.* on a scale. The naive person knows nothing of this duality, he believes that the event as observation and as a physical fact are identical. But as soon as this naive realism has been abandoned, it is clear

that the knowledge of the physical event is possible only through the mediation of the observed event. Many observed events, however, are not simple and direct guides to physical events; the earth looks flat, and yet we know that the ancients who believed that the earth was really flat were mistaken. This example teaches us not much more than that singly observed events are only part of the requirements for knowledge of real events, other parts being the systematization through reasoning of many observed events. Another example will add a new insight: we all experience ourselves as active at some times, and passive at others; *i.e.* we make things and we are moved about by forces stronger than ourselves. Such experiences are more dominant in other cultures than our own, cultures which we call animistic or magical. Now science had to fight magic in order to come into its own, and so it banished all concepts that smacked of activity and passivity. Some extremists, and they are not few in numbers nor lacking in authority and appeal, want to banish all concepts like force or causality from true science and replace them by the concepts of regular sequences and concomitant variations. But magic, whatever its value for our knowledge of the physical world may be, is first of all a psychological datum, and as such also an event in nature which cannot be discarded in a system of all knowledge. The psychologist should understand why magical connections are experienced as well as why so-called natural ones are. And then he must examine why those observed facts did mislead as signposts into the realm of physical nature. In such an examination he might well discover certain aspects which a zealous science has too radically rejected, for in magic the relationships are internal, and this very fact may be the reason for the general scepticism against all internal relationships in nature.

In short, on the level of observation the psychologist encounters the problem of describing observed facts as observed and scrutinizing their value for our knowledge of the external world. And such scrutiny will reveal that the purification of science has gone too far, and that the scientist who philosophizes about physics may temper his criticism of the scientist who creates physics and in so doing uses concepts which seem illegitimate to him in his critical hours.

In the realm of the other source of science, in the realm of reason, the psychologist has the same problems before him: to de-

scribe rational thought as an occurrence and to establish its value as an indicator of events beyond thought. And here the significance of his work is even greater; for not only has he to deal with requiredness, with internal relationships, with values, than which no more philosophically important objects exist, but he has to convince the physicist that his own fundamental conceptions must be so changed or re-interpreted as to allow the explanation of requiredness as events in nature. Thus the psychologist studying science as part of behavior makes the same kind of contribution whether he studies observation or ratiocination. He alone can, as I see it, overcome the otherwise irreconcilable difference between naturalism and spiritualism, relativism and absolutism, fact and value.

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THE USE OF PERITONEAL LAVAGE IN TREATMENT OF EXPERIMENTAL PENTOBARBITAL POISONING*

ROBERT W. LACKEY, HERBERT A. BAILEY AND ANDRES GOTH

Since peritoneal lavage has been frequently used as a means of removing accumulated metabolites in uremia and was suggested by Muirhead (1951) as a possible means of treatment in barbiturate intoxication, it was thought desirable to study its effectiveness in removal of a barbiturate from the bodies of animals poisoned with a drug of this group.

Method. Mongrel dogs were used throughout the experiment. The animals were fasted overnight, technically an important point because of the frequency of acute gastric dilatation in unfasted animals. The procedure previously outlined by Bailey, Goth, and Lackey (1951) was used in inducing intoxication and consists essentially in administering sodium pentobarbital by intravenous drip at the rate of 0.7 mg./kg./min. until the mean arterial blood pressure falls to 50 per cent of its initial level. This dose (1 absolute lethal dose, A.L.D.) is one which kills 100 per cent of untreated animals. At this point artificial respiration was instituted and infusion of lavage fluid was begun, the pentobarbital drip being continued until 50 per cent more had been given, 1.5 A.L.D.). Peritoneal lavage was accomplished with fluid of the composition suggested by Muirhead (1952), table 1. Lavage was done

TABLE 1

Composition of Lavage Fluid

Compound	Grams per liter
$NaCl$	6.0
KCl	0.2
Citric Acid	1.52
$NaH_2PO_4 \cdot H_2O$	0.09
$CaCl_2 \cdot 2H_2O$	0.13
$MgCl_2 \cdot 6H_2O$	0.21
$NaHCO_3$	2.0
Dextrose	10.0 or 30.0

with a 16-gauge needle and using one liter of fluid at each lavage. It required approximately 13 minutes for the infusion of the fluid which was left in place for one hour. As much fluid as possible was recovered and another liter was introduced. Lavage was carried out three times. On the average, of the three

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liters of fluid used, 600 to 800 ml. was not recovered. Barbiturate levels in blood and recovered lavage fluid were determined spectrophotometrically by the method of Goldbaum (1948).

Results

The results are summarized in Table 2. Of the 12 animals in the series, 10 received isosmotic solutions and in the remaining two animals (numbers 5 and 6) the solution was rendered hypertonic by increasing the glucose content to 30 gm. per liter.

TABLE 2

Data on Pentobarbital Recovery by Peritoneal Lavage

Dog No.	Pentobarbital dose Mg./Kg.	Pentobarbital dose Total Mg.	Recovered lavage fluid Vol Ml.	Recovered lavage fluid Pentobarbital Mg. per 100 Ml.	Recovered lavage fluid Pentobarbital Total Mg.	Blood pentobarbital at withdrawal of lavage fluid Mg./100 Ml.
1	82.5	1,163	830	2.5	20.7	5.0
			1,000	1.7	17.0	6.4
			950	2.6	24.7	4.5
2	111	1,154	775	3.2	24.8	8.4
			960	2.8	26.9	7.6
			1,000	2.8	28.0	6.5
3	72	878	500	2.5	12.5	6.5
			950	1.25	11.9	5.9
			1,000	1.4	14.0	4.4
4	72.2	1,042	400	1.56	6.2	5.7
			...	2.25	...	4.6
			700	1.06	7.4	4.4
5	79	964	550	2.5	13.7	4.8
			1,220	1.4	17.0	4.6
			....	0.5	...	5.0
6	86.7	1,214	625	2.12	13.2	5.06
			860	0.81	7.0	4.1
			975	0.81	7.9	2.75
7	95.5	955	590	1.66	9.8	6.32
			1,025	1.12	11.5	4.7
			1,050	1.09	11.4	3.3
8	91	1,610	150	6.47	9.7	6.0
			1,210	1.63	19.7	4.7
			440	1.0	4.4	4.4
9	79.5	1,040	800	2.4	19.2	5.6
			900	1.9	17.1	4.4
			750	1.3	9.7	..
10	95.4	1,726	800	2.6	20.8	6.0
			800	2.5	20.0	3.1
			1,000	2.4	24.0	2.4
11	105	1,858	800	3.8	30.4	7.3
			700	2.0	14.0	3.7
			1,000	1.5	15.0	2.9
12	124	2,802	270	1.65	4.4	4.9
			1,000	...	...	4.5
			1,000	1.0	10.0	4.0

These two animals were used to indicate whether or not a hypertonic solution would increase the recovery of the barbiturate. As was anticipated, this did not prove to be the case.

Determinations of the concentration of pentobarbital were made on a total of 35 samples of recovered peritoneal lavage fluid. In only one instance was the concentration greater than 5 mg./100 ml. In 32 determinations the concentration of the barbiturate in the lavage fluid was less than 3 mg. per 100 ml. The concentration of the barbiturate in the lavage fluid roughly paralleled its concentration in the blood but of course at a lower level.

In the period of time during which peritoneal lavage was carried out some barbiturate was always recovered. The maximal amount recovered from any animal in a series of 3 lavages was 6.9 per cent of the total dose having been given. From the standpoint of therapy, it appears to us that this is a rather insignificant amount though it may be argued that the removal of any amount whatever of toxicant is of importance. Since the barbiturate level in the peritoneal lavage fluid always parallels the concentration in the blood, one would expect that successive lavages would remove progressively smaller amounts of the narcotic. Because the amount recovered even with high blood levels is relatively small, it is not likely that peritoneal lavage would be a life saving measure in many instances of Nembutal poisoning. This is particularly true if the material analyzed and reported as barbiturate consists in part of metabolites of the barbiturate as was found to be the case in urine analysis by Shonle, Ketch, Kempf and Swanson (1933), Brundage and Gruber (1937), and Roth, Leifer, Hogness and Langham (1949). The apparent irregularities in the blood barbiturate levels may be explained by the cyclic variation in blood barbiturate concentrations cited by Anderson and Essex (1942).

Though every effort was made to keep the animals in a good state during the period of the experiment, no attempt was made to keep them alive throughout a long convalescent period. Because of this we are not reporting statistical data with respect to the survival of the animals. It was definitely not evident to us, however, that the lavage technique in any way changed the course of the Nembutal poisoning in these animals. For example, they did not begin spontaneous respiration earlier than did other animals, did not regain their reflexes more rapidly, nor did they

regain consciousness sooner than animals treated by artificial respriation alone.

Recently Alwall, Lindgren and Lunderquist (1952) reported favorable results with the use of ultra filtration, forced diuresis, and dialysis in treatment of phenobarbital poisoning in rabbits. They did not measure the amount of the phenobarbital actually so removed from the body. It is possible that phenobarbital poisoning is more amendable to this type of treatment than is poisoning with pentobarbital. Taking into consideration the larger dose of phenobarbital used by these authors the serum levels reported are relatively considerably higher than we have observed with pentobarbital. This might indicate a difference in distribution of the two barbiturates in the body. There is also the possibility of a difference in the extent to which the two compounds are bound by protein which would be an important determinant in the effectiveness of treatment by dialysis.

Summary. A report is made on the rate of recovery of pentobarbital by peritoneal lavage of Nembutalized dogs. The concentration of pentobarbital in the recovered fluid was small and always considerably below that of the blood. Peritoneal lavage was not found to influence the course of recovery from pentobarbital poisoning in the dog.

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ANTITUMOURAL AUTODEFENSE*

A. LIPSCHUTZ

The title of my address sounds like a paradox, with all its classical attributes so faithfully enumerated by the Dictionary of the Spanish Academy: "Something which is strange and contradictory to common opinion and to the feeling of people in general"; "an assertion which though improbable and absurd is [none the less] presented with the appearance of truth"; something rhetorical, "a mental procedure consisting in the use of expressions and sentences which involve contradiction." A paradox, inadmissible in scientific thought and expression. . . .

The tumour and especially the malignant one belongs so far, undoubtedly, to those diseases before which the human being, and so also the medical practitioner, feels rather defenseless. The therapeutic possibilities at the disposal of the surgeon and of the radiologist are limited; and even more limited and mostly nil are those at the disposal of internal medicine. Nevertheless, it is here asserted that the concept of an antitumoural autodefense is neither a paradox, nor an absurd chimera; as far as this concept is opposed to common opinion this is not sufficient to condemn it. However, we must freely admit that the concept involves contradiction. But the latter resides in the ever moving facts—and it is the purpose of the concept to put them, mentally, in due place and order, as scientific concepts in general do.

Acquired immunity against tumourigenic virus

The idea of an antitumoural autodefense is, in the first place, linked with tumours due to virus as known since the classical work of Peyton Rous of more than 40 years ago. He gave definite evidence that a spindlecell sarcoma of the chicken can be transmitted by the inoculation of a saline extract or suspension freed of tumoural cells (see summary Rous, 1943). Work with another tumour due to a virus, the transplantable cancer of the skin of the rabbit, the epithelioma of Brown-Pearce, deserves special interest

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from the point of view of antitumoural autodefense. When an emulsion of this tumour is inoculated into the brain or testicle, malignant tumoural evolution with metastases is the rule; 80 to 100 per cent of the inoculated animals die. On the contrary, a benign evolution takes place when an intradermic inoculation is made: the tumour disappears in a few weeks. Similar animals acquire immunity against a new injection even when intracerebral or intratesticular. The immunity is a specific one and apparently definite. Work done in the course of 25 years by different authorities and among them Besredka leaves no doubt about this kind of acquired antitumoural immunity (Casey, Meyers and Drysdale, 1948).

The workers of the group of Peyton Rous and among them especially Kidd have given full evidence that the blood serum of rabbits with Brown-Pearce carcinoma contains specific antibodies (summary Kidd, 1946). When carcinoma is incubated for 2 hours with the serum of these rabbits it looses transplantability. Some substance present in the growing tumor acts as an antigen. The latter is thermolabile as most of antigens are.

Variation of the tumourigenic virus due to passage in a foreign species

This new aspect of specific bodily responses has been studied by the group of Duran-Reynals. Tumours can be elicited in the duck by the filtrate of the chicken sarcoma of Rous. But the tumour growing in the duck is structurally different from the original chicken sarcoma; it is no longer a spindlecell sarcoma but lymphocytoma, various kinds of leucemia, tumours of the periosteum. It is not simply a different response of specific territories but much more than that: the virus of the chicken has undergone profound changes in the body of the duck. Thus, a cell free extract of duck tumours injected into the chicken induces not the original tumour but proliferative phenomena of a quite distinct type. Another very striking example of the changes the virus of the chicken sarcoma undergoes in the body of the duck is the following: whereas the pigeon is refractory to the original chicken virus the latter after its passage through the duck becomes capable of eliciting tumours also in the pigeon (Duran-Reynals, 1942, 1943, 1947, 1950, 1951; Borges and Duran-Reynals. 1952).

The impressive findings of Duran-Reynals and his students have been more recently corroborated by similar experiments in amphibia. The virus of renal carcinoma of the frog when inoculated into a salamander produces chondrosarcoma of the bone. When reinoculated from the salamander into the frog the virus is capable of eliciting in the latter not only renal carcinoma but also a bone tumour (Rose and Rose, 1952).

On the basis of his ample work Duran-Reynals has reached the very relevant conclusion that tumourigenic viruses behave in a manner similar to that of classical infectious viruses in so far as they acquire new tumourigenic potencies due to a passage through an hitherto foreign biological system.

Tumours due to viruses are extremely scarce in human pathology. However, these problems so strange at first sight from a purely practical point of view offer considerable clinical interest. A virus similar to that discovered by Bittner in the milk in some strains of mice with hereditary mammary adenocarcinoma and visible by electronic microscopy is apparently present also in human milk (Passey, Dmochowski, Astbury and Reed, 1950; Gross, Gessler and McCarty, 1950). Indeed, only the first steps have been made hitherto; but the findings are certainly suggestive.

Acquired immunity against transplantable tumours

Much experimental work has been done in this field in the course of years in mice and rats (Barrett, 1952; Hauschka, 1952; Loefer and Mefferd, 1952b). A tumour which takes in every animal of the pure strain in which it originated may take and grow also in some animals of a foreign strain; but invariably, after a number of weeks, involution and resorption of the tumor takes place in the latter. However, something very important has taken place in the meantime: the animals of the foreign strain have acquired immunity against the taking of the tumor when a second transplantation is made (Barrett, Hansen and Spilman, 1951). The lymphatic system is apparently implicated in this kind of autodefense: resistance against the second transplantation is diminished or abolished when the lymphatic system is blocked by cortisone (Foley and Silverstein, 1951; Foley, 1952c). Immunity can be produced also against transplantable lymphosarcoma of the own strain by lymphatic nodes, thymus or spleen of a foreign strain implanted several days before graft-

ing the lymphosarcoma (Foley, 1951a, 1952b). Resistance against transplantable tumours can be enhanced by nutritional means (Loefer and Mefferd, 1952a).

The antigen in acquired antitumoural immunity

Here a very relevant nay fundamental question arises. It is evident that the transplanted cells of the tumour of the foreign strain are the carriers of an antigen. Is this antigen due to the cell being *turoural*, or to the cell belonging to a *foreign strain?* Much work has been done on acquired resistance against transplanted tumours with previous administration of non-tumoural tissues. British investagors were the first to work, almost fifty years ago, on these lines(Bashford, Murray and Cramer, 1908). More recently it has been recognized that results depend fundamentally on the genetic conditions both of the strain to which the donor of the tumour or tissues belong, and of the strain into which they are transplanted (Gorer, 1937, 1938, 1948). These findings were the beginnings of what has been called later an "Immunogenetics." Thus, to give an example, the taking of mammary cancer A in RI mice was reduced, with the adiministration of 0.2 cc. of defibrinated blood of A, from 100 per cent to 7 per cent; on the contrary, no significant reduction was obtained with blood of C57 mice (Barrett, 1952).

These experimental results are certainly stimulating. But is it necessary to add that it would be preposterous to nourish hopes as to application in humans? And there is symbolical value in the cruel fact that with similar antigens not only increase but also *diminution* of antitumoural resistance has been produced (Snell, Cloudman and Woodworth, 1948; Kaliss and Snell, 1951; Kaliss, 1952; Kaliss and Molomut, 1952).

Hormonal imbalance and tumourigenesis

The mechanisms of autodefense both against a tumourigenic virus and a transplantable tumour apparently are similar to, though possibly not coincident with those known from classical immunology. A third aspect of antitumoural autodefense probably inherent to all mammals, is of a fundamentally different nature: it is related to the *steroid homeostasis* of the body. The concept of a similar antitumoural autodefense originated in the

course of our work with experimental tumours due to an hormonal imbalance. Its discussion brings us into immediate contact with candent problems of human pathology.

In the cat or guinea pig luteal cysts may appear in the ovarian remnant or fragment *in situ* after subtotal castration ("ovarian fragmentation"). The sexual cycle in the guinea pig is upset. A typical proliferation of the uterine glands penetrating deeply into the myometrium takes place; an epithelioma of the cervix was found in one of these animals 32 months after the operation (Lipschutz, 1936, 1937, 1938). The hypophysis of these animals when injected into mice produced a greater increase of uterine weight than with the hypophysis of normal females. It became thus evident that with subtotal castration the normal ovarian hypophyseal relationship may be overthrown; there is an hormonal imbalance caused by a primary failure of the ovary to control in due form the gonadotrophic activity of the hypophysis by its steroids. Follicular growth is then deviated from its usual course and atypical growth of ovarian and uterine structures is the result. This was the first time when tumoral growth due to an hormonal imbalance, or to a failure of the steroid homeostasis, was experimentally produced. Our findings were corroborated and amplified by several investigators. Uterine fibromyoma, fibroma (or dermoid) of the abdominal serosa, abdominal cystadenoma of Wolffian origin, and uterine subserous adenomyoma, have been described in guinea pigs with subtotal ovariectomy (Morató, 1941; Nadel, 1949; Ponse and Dovaz, 1950, 1951; Bruzzone, 1950). In one case a tumour of the renal pelvis was found at 45 months; there was also a uterine condition reminiscent of adenocarcinoma (Bruzzone, unpublished experiments).

Tumours in intrasplenic ovarian grafts

A dramatic corroboration of the concept of steroid homeostasis as a means of antitumoural autodefense has been given more recently by experiments in which one ovary was grafted into the spleen whereas the second ovary was eliminated. Experiments with intrasplenic autografts were made in guinea pigs as early as 1942. Hemorrhagic follicles appeared in these grafts, similarly to what happens in the Aschheim-Zondek pregnancy test (Lipschutz, 1946; Lipschutz, Ponce de León, Woywood and Gay, 1946). The explanation was an easy one: the whole œstro-

gen produced in the intrasplenic graft had to pass through the liver to be inactivated there, in such a way that only a small amount of œstrogen was allowed to reach the general circulation. This, and possibly also the disturbance of the rhythm of œstrogen production, apparently is responsible for the uncontrolled gonadotrophic hypophyseal activity which may continue for as long as 3 years as shown by hemorrhagic follicles still present in those old grafts. However, if sufficient quantities of œstrogen are administered no hemorrhagic follicles are produced.

Simultaneously with these experiments in the guinea pig, and quite independently of ours, Biskind and Biskind (1944) made use of intrasplenic grafts in the rat. They have been the first to describe granulosa cell tumours and luteamata in grafts. In our guinea pigs. *luteomata* appeared at about 10 months. They originated principally from cells of the ovarian stroma and of the follicular theca. The masses of lutein cells may break through the fibrous capsule which separates the ovarian graft from the spleen, and come into immediate contact with the splenic parenchym. The weight of similar luteomatous ovaries may be 50 to 100 times that of the normal gonad. At 2 to 3 years the graft may be built up almost entirely of lutein cells with no individual corpora lutea ("exclusive" luteoma; Iglesias, Mardones and Lipschutz, 1953a).

Experiments with intrasplenic grafts were very successful in mice where metastasis are produced, especially in the liver (Li, 1948; Li and Gardner, 1947; Furth and Sobel, 1947). In mice the tumour is transplantable. On the contrary, in the guinea pig we were unable to find metastasis, and all trials with transplantation failed. However, this failure may have been due to the fact that highly hybridized strains of guinea pigs have been used.

Another interesting finding in intrasplenic ovarian grafts were the nodules of Wolffian origin which are often cystical, and nodules of the Brenner type similar to, though not identical with those known in women (Iglesias, Mardones, Bruzzone and Lipschutz, 1953).

At the beginning both the Biskinds and ourselves thought that ovarian tumourigenesis in intrasplenic grafts depended on complete suppression of œstrogenic activity in the body. Later on this concept has been shown to be erroneous (Lacour, Oberling and

Guérin, 1951; Iglesias, Mardones and Lipschutz, 1953b). Thus, ovarian luteomata and tumours of the Brenner type were present in our work in animals in which œstrogen circulated in amounts sufficient to cause an abnormal increase of uterine weight, cystical glandular hyperplasia of the endometrium and metaplasia.

The Law of the Sex Rhythm

After all this there is now full evidence that ovarian and extraovarian tumours can be elicited by an abnormal timing in the production, liberation and metabolism of ovarian steroids in the body. In other words: ovarian and extraovarian tumours are produced by a deviation of the ovarian rhythm. The latter has been shown to be paramount for antitumoural autodefense in even a more striking manner in experiments very easy to duplicate. Fibroids, or dermoids, and fibromioma of the abdominal serosa can be elicited by a prolonged administration of œstrogens. But these tumours arise only when œstrongenic action is a continuous one: with the discontinuous administration of œstrogen. say when in the guinea pig 2 weeks of injections are followed by 2 weeks without injections, fibroids do not occur, even then when the experiment is prolonged for as much as one year (Lipschutz, Rodríguez and Vargas, 1942). Likewise, tumours do not occur if continuous administration of œstrogen is combined with rhythmic administration of progesterone (Iglesias, Lipschutz and Nieto, 1943) by which the tumourigenic action of the œstrogen is known to be antagonized or prevented (summary Lipschutz, 1950).

The sexual cycle in mammals with its rhythmic increase of œstrogen and rhythmic production of progesterone acquires a new and quite unexpected aspect: the sexual cycle is undoubtedly one of the essential means of antitumoural autodefense! It was only thanks to the *rhythmic* interplay of steroids that the body of the mammals became able in the course of organic evolution to apply in its economy substances which like œstrogens are potential carcinogens; it is only thanks to the sexual cycle or thanks to *rhythmic* production, liberation and metabolization of ovarian steroids that the body became able to maintain the steroid homeostasis which is subject to transitory changes for the very sake of an organic function so fundamental as reproduction.

The sexual cycle is in suspense during pregnancy when very deepgoing changes of the steroid homeostasis take place. Production both of œstrogen and progesterone is at its climax. Foulds (1952) in London made the very important statement that in certain strains of mice mammary adenocarcinoma occurs during pregnancy; but these nodules which appeared in the second half of pregnancy dissappear again after delivery—to reappear in the next pregnancy. Transitory mammary adenocarcinoma concommitant with transitory changes of the steroid homeostasis! Transitory neoplasm, transitory carcinoma: these are notions really heretical from the classical point of view of autonomous and irreversible neoplastic growth. But on the other hand these new notions due to the remarkable work of Foulds are in full agreement with what we know nowadays about œstrogen-induced fibroids of the abdominal serosa whose growth may be invasive and destructive without being autonomous; in agreement also with what we know since the outstanding work of Higgins on prostatic cancer in man.

We must refer here also to the new impulse given to knowledge on the sexual rhythm by the work of Harris in Cambridge. The hypothalamus has been shown to be one of the interdependent organs on whose interplay the Law of the Sexual Rhythm rests (Harris, 1949; Harris and Jacobsohn, 1952). The ovary with its 2 steroid hormones, the adenohypophysis with its 3 gonadotrophic hormones, and the hypothalamus connected with the hypophysis by a portal system "en miniature"—they all share in establishing, maintaining and readjusting the steroid homeostasis in the body. But hypothalamus means implicitly also the cerebral cortex with which it is connected by nervous pathways; hypothalamus means thus contact with ambiental stimuli. It is no more presumptuous or fantastic to admit that the latter may influence and deviate the steroid homeostasis and possibly share in the responsibility for certain types of tumourigenesis.

The liver and the suprarenal in antitumoural autodefense

The liver is known to partake in the steroid metabolism by its inactivating œstrogen, progesterone, desoxycorticosterone, testosterone. But this faculty has been shown to be functionally variable: inactivation of œstrogens may gradually increase when large quantities of this steroid pass for a sufficient length of

time through the liver (Lipschutz 1950). It is a kind of functional adaptation for the very sake of the maintenance of the steroid homeostasis.

A paramount place in the maintenance of the steroid homeostasis and in antitumoural autodefense belongs to the *suprarenal cortex*. It is so-to-say the "productive" center of the steroid metabolism in the body. No less than 29 different steroids, or corticoids, have been found up to now in the suprarenal cortex (Reichstein, 1951). Resistance against transplantable leucemia and lymphosarcoma in the rat is dependent on the presence of corticoids in the body: resistance diminishes after adrenalectomy (Murphy and Sturm, 1943; Sturm and Murphy, 1944; Stasney, Paschkis and Cantarow, 1951). The same is true for lymphatic tumours induced in mice by X-rays (Kaplan and Brown, 1951).

The assumption that the suprarenal cortex is an important factor in antitumoural autodefense is based also on numerous statements about the antitumourigenic action of corticoids. They are undoubtedly the most potent antitumourigenic steroids. The œstrogen-induced fibroids of the abdominal serosa can be prevented not only by progesterone but also by desoxycorticosterone (Lipschutz, Vargas and Núñez, 1951). Transplantable lymphatic tumours and leucemia in rats and mice can be prevented by various corticoids (Stock, Karnofsky and Sugiuru, 1951; Stock 1952). Some observations of ours led also to the assumption that prolonged administration of the tumourigenic œstrogen stimulated production or liberation of antitumourigenic corticoids (Lipschutz, 1950, p. 194).

The findings of Dobriner and associates are amply known: 17-keto-steroids which are not present in the urine of normals appear in the urine of cancer patients; one of these steroids, 11-hydroxy-etiocholanone, is probably a metabolite of cortisone (Dobriner, 1952). Indeed, none of the abnormal 17-keto-steroids is specific for cancer; they may be found also in the urine of non-cancerous patients. But this is not contrary to the concept of Dobriner that cancer is in some way related to a functional deviation of the suprarenal cortex.

Epilogue

Vast labour has been invested in the experimental study of antitumoural autodefense. One is entitled to ask whether some

profit for the treatment of cancer has arisen from all this work.

Nothing of what we know on antibodies due to tumourigenic viruses or to transplantable tumours can be applied to humans though indeed the findings are of fundamental interest for pathology. On the contrary, the new data which have been acquired about steroids as tumourigenic and antitumourigenic factors, about steriod homeostasis and about its importance for antitumoural autodefense are of an immediate clinical interest. A glimpse into journals dedicated to clinical endocrinology, metabolism, gynecology and cancer are convincing as to this. No clinical discussion on scientific lines is nowadays possible without taking into consideration those experimental factors which have been summarized in the concept of steroid homeostasis as a means of antitumoural autodefense.

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APPLICATION OF NEOTETRAZOLIUM TO OUTGROWTHS OF HUMAN NEOPLASMS IN TISSUE CULTURE

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Reports are available in the literature on the use of tetrazolium compounds in various phases of biological work. These have been comprehensively reviewed in papers by Smith (1951) and by Black, Zweifach and Speer (1953). In the study of neoplasms, observations on the effect of the various tetrazolium salts have been confined to their application *in vivo*, in homogenates, tissue particles or slices and in smears. Tissue culture was used by Stein and Gerarde (1950) who worked with chick heart fibroblasts and also by Weiss and Fawcett (1953) who employed chicken monocytes. The results of both of these investigations were negative. No reports are available on the effect of tetrazolium compounds on human neoplasms grown *in vitro* and studies of this type seemed therefore desirable.

The principle underlying the use of these salts is their reduction to water-insoluble pigments (formazans) by the action of enzyme systems. They may be employed in order to demonstrate dehydrogenase activity in general or to reveal the effect of specific enzymes, particularly that of succinic dehydrogenase. The demonstration of this specific dehydrogenase was described by Seligman and Rutenburg (1951) and a similar method was published by Foraker and Denham (1953). In the present investigation, preliminary tests using diphenyltetrazolium-chloride and neotetrazolium without any specific substrate proved unsuccessful while satisfactory results were obtained after succinate had been added. The project was subsequently defined as the adaptation of tissue culture methods to the use of neotetrazolium-succinate mixtures.

Material and Methods. The material was removed from the patients for purposes of biopsy, surgical treatment, paracentesis or thoracocentesis. Sterile precautions were observed throughout. Most cultures were prepared within

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the first two hours after removal of the specimen, but in a few instances there was a delay up to five hours. The tissues were cut into pieces not exceeding 1 mm. in diameter which were placed in Gey's solution. Coverslips (No. 1 thickness, 22 mm. square) were prepared with a drop of chicken plasma-penicillin mixture (1 cc. of plasma contains 10,000 units of sodium penicillin), and one piece of tissue was placed on each coverslip. A drop of extract from 9-day chick embryos was added, the drops were mixed by stirring and the planted piece was oriented in the center. After clotting was completed the preparation was covered with two to four drops of human cord blood serum. The coverslips were then inverted and sealed with paraffin to depression slides of 4 mm. thickness. The cultures were incubated at 37.4° and were examined daily under the microscope. In the successful cases, initial outgrowth was usually established within two days. The coverslips were then removed from the slides and transferred to a mixture of neotetrazolium and sodium succinate prepared after the method of Foraker and Denham (1953). Incubation in this solution was carried out at 37.4° for four hours. The preparations were briefly washed in Gey's solution and were then mounted in glycerin.

Results

Observations were limited to the cells of the outgrowths since the planted pieces usually stained diffusely and lacked sufficient cytological detail. The formazan deposits appeared red or dark purple and the cell borders as well as the nuclear membranes were sharply outlined. The pigment particles lay in the cytoplasm and often occupied only a portion of the cell, being lined up on one side of the nucleus. They were not found in all the cells of the outgrowth and were always absent in the slender spindle cells representing fibroblastic proliferation. Two different types of deposits were discernible: a) Purple, rod-shaped crytals which were best demonstrated with ordinary illumination, and b) pink or red droplets which could be brought out particularly by the use of phase contrast.

Outgrowths from 70 different specimens were examined by the method described. Of these, 32 represented malignant tumors as verified by histological examination, while the remainder included benign growths and lesions not of neoplastic character.

In recording the results, the first 40 cases were graded according to the amount of colored matter found in the cells of the outgrowths, regardless of whether crystals or droplets accounted for the coloration. The presence of many large particles was designated "two plus," and occurrence of occasional small deposits was considered "one plus," while absence of pigment was classi-

fied as "negative." The remaining 30 specimens were similarly graded, but in addition, the presence or absence of crystals was listed in each case. The results of each group are listed in tables I and II.

TABLE 1

Final classification of specimens	Tissue culture number	Results
Malignant:		
Carcinoma of breast	203, 208, 263	+, ++, ++
Ascitic fluid with carcinoma cells	213, 220, 223, 224, 257	++, ++, ++, +, ++
Chest fluid with carcinoma cells	197	+
Metastasis to omentum from carcinoma of ovary	206, 233	+, ++
Lymphosarcoma of lymphnodes	191, 209, 253	+, +, ++
Wilms tumor, metastatic to pleura	182	++
Carcinoma of thyroid	183	++
Neuroblastoma of adrenal	201	+
Squamous cell carcinoma of lip	237	−
Cortical cell carcinoma of adrenal	239	++
Papillary carcinoma of urinary bladder	243	+
Botryoid sarcoma of urinary bladder	241	++
Multiple myeloma of rib	262	++
Benign:		
Adenoma of thyroid	190, 210, 264, 265	+, ++, −, −
Fibroadenoma of breast	207, 240, 256, 258	+, −, −, −
Hyperplasia of lymphnode	259	++
Neurilemoma	186	−
Xanthoma of bone	189	++
Intraductal papilloma of breast	198	−
Hashimoto's struma	219, 236	−, −
Ascitic fluid without demonstrable tumor cells	202, 204, 218	+, −, ++
Chronic cystic mastitis	212	+

Results observed in 40 cultures treated with the neotetrazolium-succinate mixture and graded according to the amount of colored matter found in the cells of the outgrowths. +: "one plus"; ++: "two plus"; −: "negative".

Discussion. The results of this investigation are consistent with the previous observations, published by different authors working with human neoplasms, on the effect of the tetrazolium compounds upon tissue particles or slices (Antopol, Glaubach & Goldman, 1948; Black & Kleiner, 1951; Black, Opler & Speer, 1950; Black, Zweifach & Speer, 1953; Calcutt, 1952; Foraker & Denham, 1953; Hsu & Hoch-Ligeti, 1953; Straus, Cheronis & Straus, 1948), *in vivo* (Bunge, 1951; Schuermann, 1950; Straus, Cheronis & Straus, 1948) and in homogenates (Black, Zweifach & Speer, 1950; Rutenburg, Gofstein & Seligman, 1950). A spe-

cific advantage of the tissue culture method lies in its adaptability to a detailed high-power examination of individual undisturbed cells with regard to the size and distribution of the formazan deposits. Absence of tetrazolium reduction in thin tissue sections was reported by Seligman and Rutenburg (1951) and was confirmed by Black, Zweifach & Speer (1953). These authors explain this failure by the fact that the cells are damaged in the preparation of thin slices. Although thinner sections can be made from larger blocks after incubation with tetrazolium, there is still the unknown factor of changes produced by slicing, freezing and fixation. All of these difficulties are overcome by the tissue culture method which, in addition, produces larger cells suitable for phase-contrast microscopy and photography.

TABLE 2

Final classification of specimens	Tissue culture number	Results
Malignant:		
Carcinoma of breast	277, 308	+(c), +(c)
Lymphosarcoma of mesentery	292	++(c)
Squamous cell carcinoma, metastatic to lymphnode	298	++(c)
Ascitic fluid with carcinoma cells	300	++(c)
Adenocarcinoma of uterus	306	++(c)
Carcinoma of pancreas	310	+(c)
Botryoid sarcoma of urinary bladder (recurrence)	319	++(c)
Undifferentiated carcinoma, metastatic to lymphnode	332	++(c)
Carcinoma of stomach	353	++(c)
Benign:		
Hyperplasia of lymphnode	268, 271, 274, 295, 329, 285	++, +, −, −, +, +
Normal endometrium (proliferative stage)	280, 286, 288, 334	++(c), ++(c), ++(c), ++(c)
Adenoma of thyroid	283, 325	+(c), +(c)
Fibroadenoma of breast	276	−
Fibroma of ovary	355	−
Papilloma of nasal mucosa	293	++(c)
Sclerosing adenosis of breast	294	+(c)
Hyperplasia of thyroid	284	+(c)
Neurilemoma	302	−
Intraductal papilloma of breast	311	++
Endometrial polyp	327	++(c)

Results of neotetrazolium-succinate treatment of 30 cultures. Grading is performed on the same basis as in Table I, but the findings are further qualified in each case by the presence or absence of crystals. +: "one plus"; ++: "two plus"; −: "negative"; (c): "crystals present".

The possible objection that the pigment deposits might represent artifacts or fragments of non-specific matter deserves care-

ful consideration. The granularity of the cytoplasm that can be observed in unstained preparations of tissue cultures, particularly under phase-contrast, is differentiated from the formazan particles by the size and distribution of the granules and also by the absence of the purple or red color. The droplet type of deposit may be caused by accumulation of the dye in fat vacuoles due to the solubility of the formazan in lipid solvents. This explanation has been emphasized by Black, Opler & Speer (1950), by Seligman & Rutenburg (1951) and by Rutenburg, Wolman & Seligman (1953). The importance of noting the absence or presence of actual formazan crystals, as shown in Table II, is therefore evident. Another potential source of false conclusions is suggested by the observations of Straus, Cheronis & Straus (1948) who found that the tetrazolium compounds were reduced more rapidly in regions where there was a local deficiency in oxygen. Such a condition may exist in tissues grown *in vitro* and it is often difficult to determine whether or not the cells are in a state of necrobiosis which would favor anoxia. An attempt was made to overcome this particular problem by staining the preparations as soon as initial outgrowth was observed.

Recently, doubts have been expressed that the appearance of the formazan is an accurate indicator of the site of succinic dehydrogenase (Novikoff, 1953; Farber, 1953). Other enzyme systems may be responsible, even when succinate is used as a substrate. A negative reaction may not necessarily indicate absence of the enzyme within the cell but could be due to failure of the large tetrazolium molecule to pass through the cell membrane (Weiss & Fawcett, 1953). In view of these possible sources of error, the here presented findings are considered representative of the neotetrazolium effect on cells grown *in vitro* rather than of the succinic dehydrogenase activity within these cells.

In spite of the outlined difficulties involving the interpretation of results, there are definite advantages in using the here reported method. The most apparent ones are that formazan deposition can be examined in individual intact cells and that the effect favors the study of cytological details. The method is well suited for an investigation into the spatial relationship between the formazan deposits and mitochondria, also between these deposits and intracellular lipids or exogenous particulate matter. Studies to this effect are being carried out by the author with the use of

Janus green, osmic acid, Sudan black, neutral red and brilliant cresyl blue.

One of the aims of most procedures using tetrazolium salts is the detection of patterns in the formazan deposition that might be specific for cells of malignant tumors. The majority of reports dealing with observations on tissue slices, homogenates and smears have so far failed to reaveal any such specificity. There are some exceptions such as the results of Straus, Cheronis & Straus (1948) who found that carcinomas reduced tetrazolium chloride while the surrounding tissue remained unstained. Mackenzie and Fuller (1950) also observed a difference between benign and malignant cases in tetrazolium-treated smears made from vaginal secretions and cervical scrapings.

In the here presented observations, positive results were obtained with almost all of the 32 malignant tumors which had produced outgrowths in tissue culture. A notable exception was a squamous cell carcinoma of the lip (TC No. 237) that failed to form any colored deposits. It is possible that the large quantity of keratin granules present within this tumor might have obscured the formazan particles in this case. The fact that results of the "two plus" type were furnished by some benign tumors and essentially normal tissues indicates that the reaction, in its present form, cannot be considered specific for malignancy. However, when tabulation of the findings is qualified by the presence or absence of formazan crystals, as indicated in table II, "two plus" results in the benign group become limited to a few instances with the exception of normal endometrium which was strongly positive in all instances. It seems likely that a positive outcome depends on the ability of the tissue to grow at a relatively rapid rate and that, on this basis, the difference between benign and malignant tumors is more apparent than that between neoplasms and certain normal tissues. The absence of appreciable quantities of formazan in fibroblasts can be used to advantage in the evaluation of findings obtained by tissue culture since fibroblastic outgrowth is often obtained from lesions not neoplastic in character as well as from many benign tumors. Finally, failure of the reaction in the absence of active outgrowth is helpful in cases which are doubtful as to whether or not the *in vitro* method has been successful.

Summary. Outgrowths from tissue cultures of 70 surgical specimens, including 32 malignant tumors, were incubated in a mixture of neotetrazolium and sodium succinate. Varying degrees of formazan deposition were observed and were graded as "one plus," "two plus" and "negative." Positive results were demonstrated for most of the malignant tumors and also for some specimens of the benign group, The advantages gained from applying this procedure to cells grown *in vitro* lie mainly in the demonstration of formazan deposits within intact cells without any previous sectioning or fixation and in the recognition, by absence of pigment formation, of purely fibroblastic proliferation or of failure to obtain any outgrowth. Differences between the benign and malignant groups become more apparent when the results are given in terms of presence or absence of formazan crystals rather than of pigmented deposits in droplet form.

The author expresses his grateful appreciation to Dr. C. M. Pomerat of The University of Texas Medical Branch for his invaluable advice on matters of tissue-culture technique. He is also indebted to Mrs. Adeline Green, M.T., and to Mrs. Jacqueline Carlos, M.T., for their technical assistance.

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LEGENDS TO ILLUSTRATIONS

FIGURES 1 TO 8. Photomicrographs of outgrowths treated with neotetrazolium-succinate mixture representing "two plus" results. Of note are the formazan particles which appear black in the illustrations but are actually dark red or purple. In Figures 1 and 2 the deposits are crystalline in character, in Figures 3 to 6 they are of a mixed type, and Figures 7 and 8 show droplets only.

FIG. 1. Adenocarcinoma of stomach. T.C. 353 (x 970).

FIG. 2. Undifferentiated carcinoma, metastatic to retroperitoneal lymph-node. T.C. 332 (x 970).

FIG. 3. Normal endometrium in proliferative stage. T.C. 334 (x 970).

FIG. 4. Squamous cell carcinoma, metastatic to cervical lymphnode. T.C. 298 (x 970).

FIG. 5. Same case as Fig. 4. (x 100, phase contrast.)

FIG. 6. Carcinoma of breast. T.C. 208 (x 970, phase contrast).

FIG. 7. Follicular carcinoma of thyroid. T.C. 183 (x 970, phase contrast).

FIG. 8. Xanthoma of rib. T.C. 189 (x 430, phase contrast). This lesion was considered a variant of fibrous dysplasia and contained large quantities of lipid matter.

FIGURES 9 AND 10. Treated outgrowths representing "one plus" results. Figure 9 shows the crystalline type and Figure 10 the droplet variety.

FIG. 9. Adenoma of thyroid. T.C. 283 (x 970).

FIG. 10. Lymphosarcoma of inguinal lymphnode. T.C. 191 (x 970, phase contrast).

FIGURES 11 AND 12. Treated outgrowths showing negative results. The granularity of the cytoplasm is emphasized by the phase contrast, but no pigment deposits are seen.

FIG. 11. Intraductal papilloma of breast. T.C. 198 (x 970, phase contrast).

FIG. 12. Sediment of ascitic fluid without demonstrable tumor cells. T.C. 204 (x 970, phase contrast).

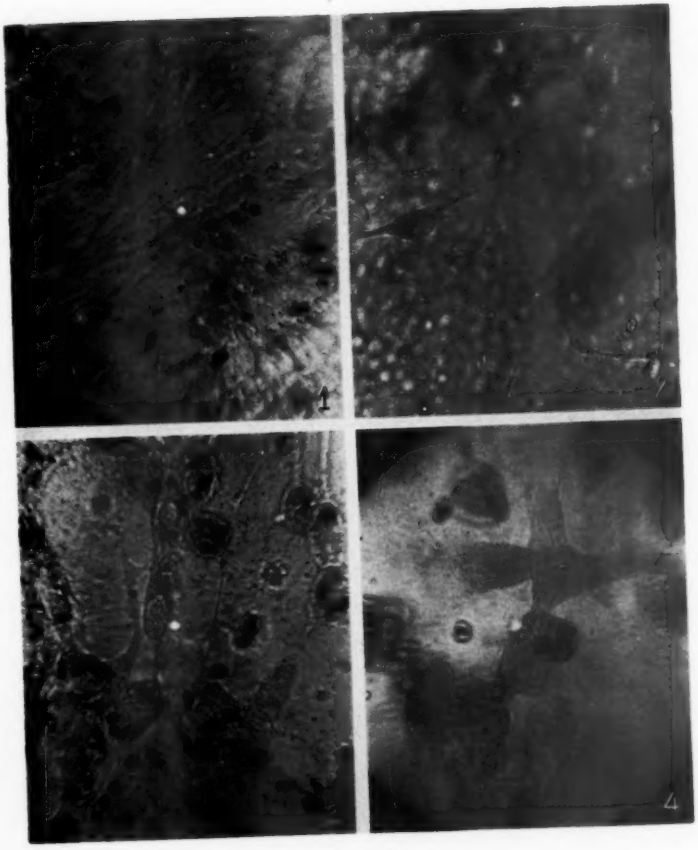

FIGURE 1–4

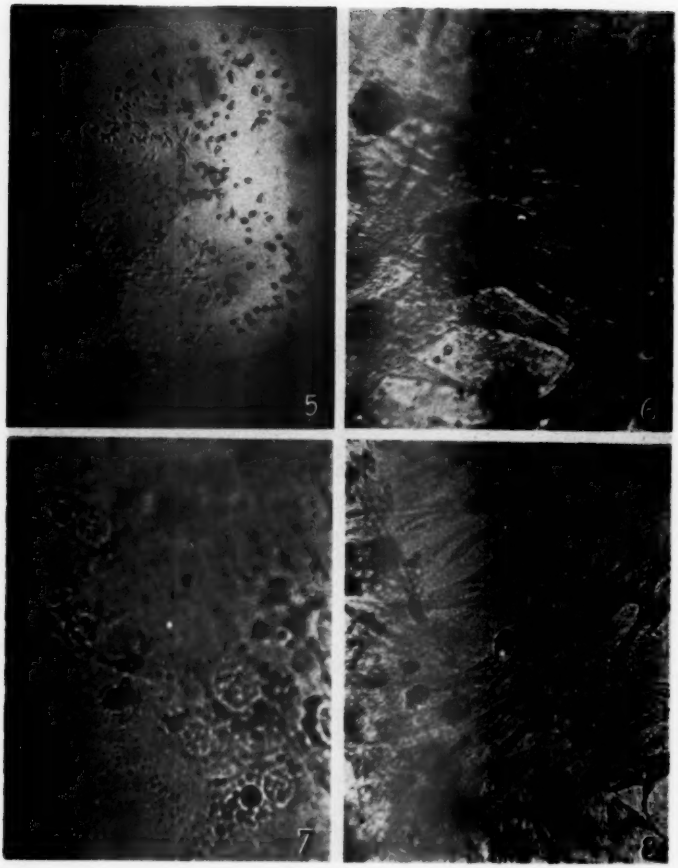

FIGURE 5–8

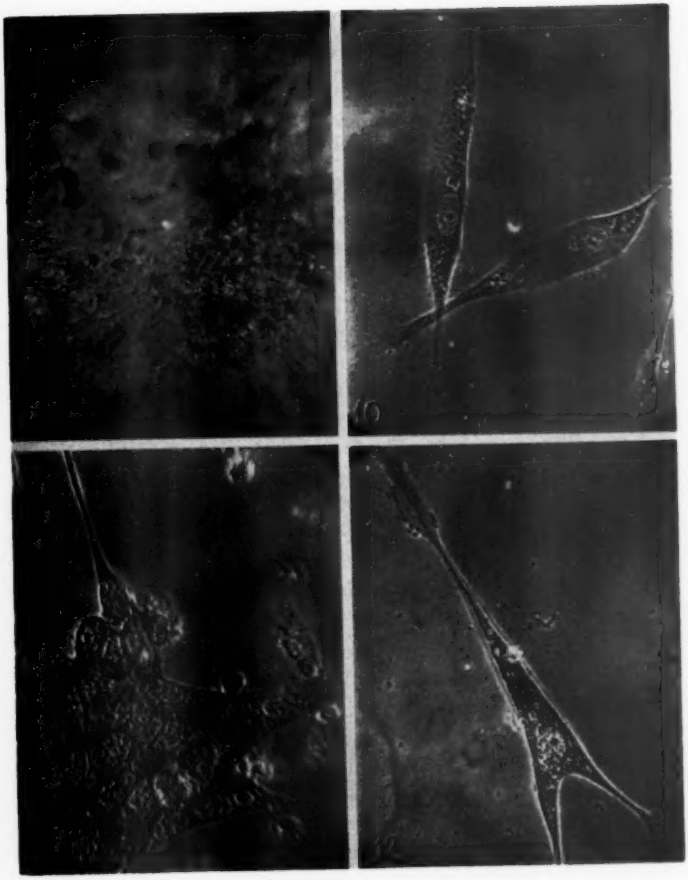

FIGURE 9–12

SEROLOGICAL REACTIONS IN RHEUMATOID ARTHRITIS

V. The Agglutination of Sensitized Human Group O Erythrocytes by Rheumatoid Arthritis Serum*

Robert M. Pike, S. Edward Sulkin and Robin I. Burdette

The serum of many patients with rheumatoid arthritis agglutinates not only sensitized sheep cells (Waaler, 1940; Rose *et al.*, 1948) but also the erythrocytes of man and other animals if sensitized with certain immune sera (Pike *et al.*, 1949; Wager, 1950). This property of agglutination enhancement is also exhibited by normal human serum and the serum of normal animals (Pike *et al.*, 1951) but to a lesser degree than by the serum in rheumatoid arthritis. Agglutinins for unsensitized sheep cells are present in most human sera. These agglutinins can be absorbed without removing the factor that causes agglutination of sensitized cells (Pike *et al.*, 1949; Wager, 1950; Jawetz and Hook, 1949; Heller *et al.*, 1949). If erythrocytes for which human serum contains no agglutinins could be substituted for sheep cells, there would be no necessity for preliminary absorption. Human O cells would be expected to fulfill this qualification at least with most human sera.

Wager (1950) showed that sensitized human O cells were agglutinated in rheumatoid arthritis serum (RAS). Since one initial attempt to demonstrate the agglutination enhancing factor using O cells was unsuccessful (Pike *et al.*, 1949) and since the rabbit anti-O cell serum used was obtained after a brief period of immunization, it seemed that the discrepancy might be resolved by immunizing several rabbits with human O cells and determining the sensitizing power of the rabbit serum at successive stages of immunization. This report concerns the results of this experiment together with similar observations on the sera of rabbits immunized with sheep cells.

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* From the Department of Bacteriology and Immunology, Southwestern Medical School of The University of Texas, Dallas. This work was aided in part by a grant from the Rose Lampert Graff Foundation, Los Angeles, California. Received for publication November 2, 1953.

Materials and Methods

Erythrocytes. Human and sheep blood was stored in Alsever's solution unless otherwise specified. Cells were washed 3 times in saline before use. The final concentration of cells in the agglutination test was 0.25 per cent.

Human serum. In order to provide a constant reagent for use throughout these experiments serum from patients with rheumatoid arthritis was pooled and stored at —20° C. without preservative and was inactivated at 56° C. for 30 minutes before use in a test. Normal sheep cell agglutinins were absorbed as previously described (Pike *et al.*, 1953).

Antierythrocyte serum. Rabbits were immunized by the weekly intravenous injection of 0.5 ml. of washed, packed human O or sheep cells suspended in a convenient volume of saline. The agglutinin titer of the immune serum was determined by adding 0.5 ml. of 0.5 per cent cells to each tube of a series of dilutions of serum made in 0.5 ml. of saline. In preliminary tests the dilutions were doubled in successive tubes. In final titrations the dilutions were increased by increments of 1,000. A fresh pipette was used for each dilution since the titers obtained were used in calculating the sensitizing dose of serum to be used later.

Test for agglutination of sensitized cells. Serial doubled dilutions of RAS or normal serum were prepared in 0.5 ml. of saline. To each tube was added 0.5 ml. of 0.5 per cent sensitized cells. Sensitized cells were prepared by combining 10 per cent cells and diluted immune serum in the proportions required to give the desired concentrartion of cells and the desired fraction of the minimal agglutinating amount of serum. All tests were incubated at 37° C. for 1 hour, placed in the refrigerator overnight and read while cold.

Experimental Observations

Sensitizing power of rabbit antihuman O cell serum during immunization. Eight rabbits were given weekly injections of human O cells. Immediately before each injection a sample of blood was obtained from the ear of each rabbit. The serum from each sample was stored at —20° C. until the completion of the immunization when all samples were tested at the same time under the same conditions. The titer of each serum for human O cells was determined. On the basis of this titration cells were sensitized with ½, ¼ and ⅛ aggulutinating unit (minimal aggulutinating amount) and tested against serial dilutions of pooled RAS. The results are shown in Figure 1. Each point on the graph represents the geometric mean of the titers of the 8 rabbits. The mean titer for unsensitized cells increased in the expected manner during immunization. Although the first injection produced a mean titer of 80, cells sensitized with ½ of this amount of serum

were in no instance agglutinated by RAS in a dilution of 1 in 20. Following the second injection, however, the serum attained the capacity to sensitize cells to agglutination in RAS. This sensitizing power continued to increase slightly with additional injections. Cells sensitized with $\frac{1}{8}$ agglutinating unit of the serum of each rabbit, were not agglutinated at any stage of the immunization. Two points are evident in the results of this experiment: (1) the sensitizing capacity of the rabbit serum in terms of the agglutinating unit for unsensitized cells increased during immunization and (2) sensitizing capacity was rapidly lost on dilution.

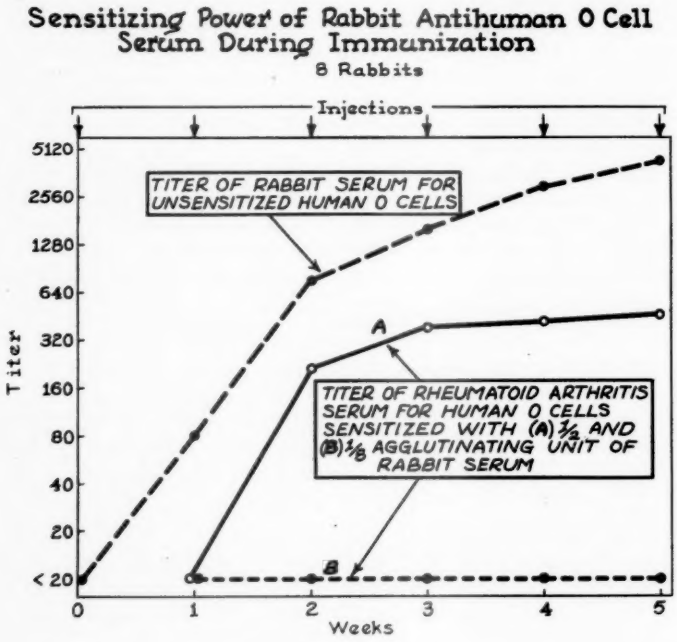

FIGURE 1

Sensitizing power of rabbit antisheep cell serum during immunization. For the purpose of comparison an experiment was undertaken similar to the preceding one but using sheep cells. Three rabbits were immunized and bled one week following each injection. The titer of each sample of serum for sheep cells was

determined as well as the titer of pooled RAS for sheep cells sensitized with each sample of serum. Figure 2 shows the geometric mean titers during the course of immunization. Following a single injection of sheep cells the rabbit sera showed some sensitizing power when used at ½ the agglutinating unit. As in the experiment with human O cells, the sensitizing capacity increased following the second and third injection. After the fourth injection the sensitizing power decreased in spite of a continued rise in the agglutinin titer. This decrease was exhibited by all three rabbits.

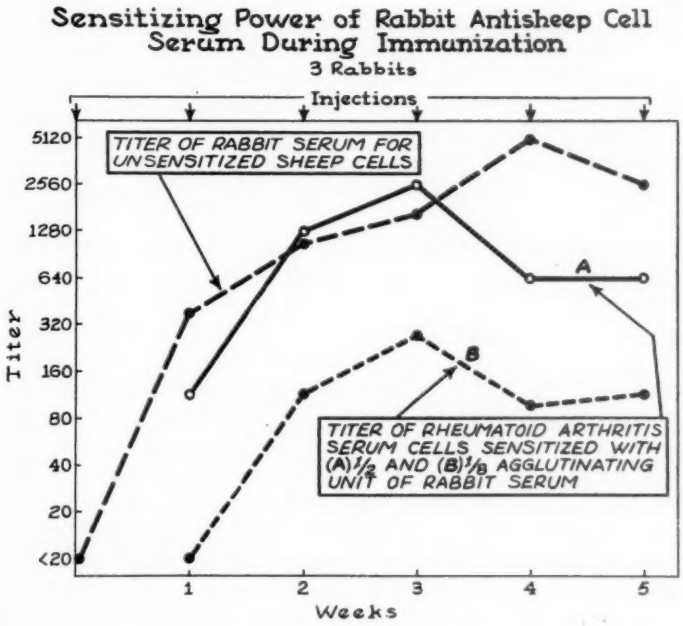

FIGURE 2

Figures 1 and 2 also show certain differences between the reactions with human O and sheep cells. The pooled RAS agglutinated sensitized sheep cells to higher titers than sensitized O cells. Furthermore, the sensitizing power of rabbit antisheep cell serum disappeared less rapidly on dilution; cells sensitized

with ⅛ agglutinating unit of serum obtained after two or more injections were agglutinated to significant titers.

The rate of decrease in sensitizing capacity on dilution is more clearly shown in Figure 3. Each point on the graph represents the titer of RAS for cells sensitized with an individual sample of rabbit serum regardless of the stage of immunization. The lines are drawn through points corresponding to the geometric mean titers for all the rabbit sera tested at each sensitizing dose. The decrease in sensitizing capacity on dilution was more rapid when human O cells were used than when the sheep cell system was employed.

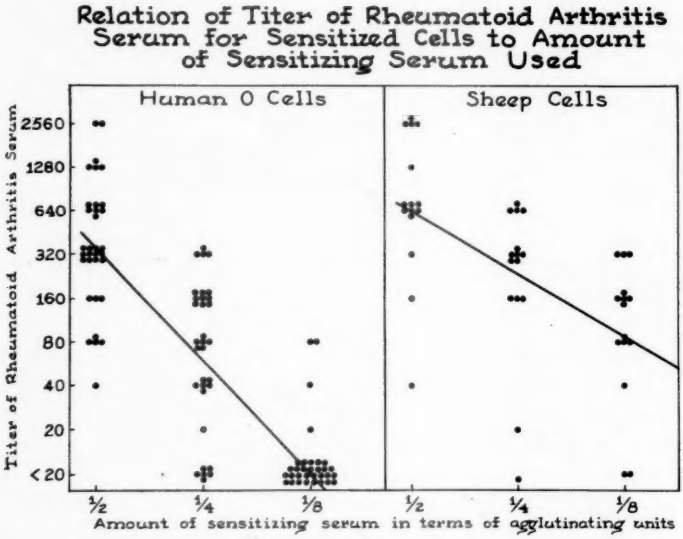

FIGURE 3

Discussion. These observations confirm the report of Wager (1950) that human O cells sensitized with homologous immune rabbit serum may be agglutinated by RAS. It is probable that the erythrocytes of any species similarly sensitized would be agglutinated by RAS containing the agglutination enhancing factor. It has already been shown that, in addition to sheep and human O cells the factor enhances the agglutination of guinea pig (Wager,

1950; Hobson and Gorrill, 1952) chicken and human A (Wager, 1950), goat (Pike *et al.*, 1949), mouse (Hobson and Gorrill, 1952) and beef (Pike *et al.*, 1949) cells by homologous immune rabbit serum as well as the agglutination of sheep cells by homologous immune serum prepared in the guinea pig (Wager, 1950) and in the horse (Hobson and Gorrill, 1952). On the other hand no agglutination enhancement was shown in the case of sheep cells in infectious mononucleosis serum (Pike *et al.*, 1949; Wager, 1950) Rh positive cells in human serum containing Rh agglutininins (Wager, 1950), human B cells in group A serum (Wager, 1950) or various erythrocytes in the presence of agglutinins occurring normally in the sera of heterologous species (Pike *et al.*, 1949; Wager, 1950). It appears, therefore, that these two categories of agglutinins differ with respect to their capacity to sensitize cells for agglutination in RAS. These experiments show that agglutinins appearing early in the process of immunization are deficient in their capacity to sensitize. The characteristics of the agglutinins upon which these differences in sensitizing power depend are not apparent. The results indicate, however, that agglutinins which appear soon after the antigenic stimulation differ qualitatively from those appearing later.

The fact that sensitized human O cells may be agglutinated by RAS suggests the possibility of using these cells in a practical test to aid in the diagnosis of rheumatoid arthritis. The absence of agglutinins for unsensitized human O cells in most human sera is a distinct advantage. On the other hand, the rapid decrease in sensitizing capacity of rabbit antihuman O serum on dilution makes it necessary to sensitize with an amount of serum which is near the smallest amount causing agglutination. A test with human O cells is, therefore, less sensitive and gives less consistent results than the test employing sheep cells (Pike *et al.*, 1952).

Summary. The observation that the agglutination of human O cells by homologous immune rabbit serum may be enhanced by serum from patients with rheumatoid arthritis has been confirmed. Rheumatoid arthritis serum, however, had a greater effect on agglutination caused by rabbit serum obtained late in the process of immunization than on serum drawn after a single injection of human O cells. A similar but less marked increase in sensitizing capacity during the course of immunization was noted when rabbits were immunized with sheep cells. The sensi-

tizing capacity of rabbit antihuman O cell serum decreased more rapidly on dilution than did the sensitizing capacity of rabbit antisheep cell serum.

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EMERGENCY USE OF ANTIBIOTICS IN THE TREATMENT OF WOUNDS*

ROBERTSON PRATT AND JEAN DUFRENOY

Use of any drug always entails a calculated risk. The anticipated benefits to be gained through use of the drug must be weighed against the potential hazards that may arise from its use. This is true even of the antibiotics, popular notions of "miracle" drugs notwithstanding, and is especially true in the treatment of wounds. Since the time of Pasteur and Lister and the classic observations of the latter on carbolic acid, it has beeen known that in many instances antiseptics hinder rather than hasten the healing of wounds, and the emphasis has been more an asepsis than on antisepsis.

A physiologic explanation for the unfavorable effects of some chemicals on wounds became available when the phenomenon of phagocytosis was discovered, and today it is recognized that the best guardians of wounds are the leukocytes. Therefore, whenever use of a drug is contemplated in the treatment of a wound, the effect of that drug on leukocytes and on phagocytic activity must be evaluated. Fortunately, often it is possible by judicious use of appropriate antibiotics and of other suitable medicaments to provide helpful reinforcement for the natural defense and repair mechanisms of the body and its tissues.

Prophylaxis vs. Chemotherapy of Wounds.—Emergency use of antibiotics for the treatment of wounds may be considered from two points of view; namely, prophylactic to prevent local or systemic infection from developing and therapeutic to hasten resolution of an established infection. Selection of the route of administration, dosage, and pharmaceutical form of antibiotic and, in some instances, even of the type of antibiotic itself may depend on whether the medication is meant to be preventive or curative. For example, in chemotherapy the choice of an antibiotic is made according to the kind of microorganism responsible

* From the University of California College of Pharmacy, San Francisco. Based on one of a series of lectures on "Emergency Medical and Health Services" co-sponsored by the School of Public Health and the Extension Division of the University of California, Berkeley, California, September, 1953.

for the infection and there are definite symptomatic criteria whereby the efficacy of the treatment may be determined. If the infection is due to Gram-positive organisms, some form of penicillin generally is first choice; if the infection is due to Gram-negative bacteria, Aureomycin[1] or Terramycin[2] usually is selected. In either instance the adequacy of the treatment can be evaluated in terms of fall of temperature or remission of other clinical symptoms of infection.

Prophylaxis, on the other hand, must be based on an estimate of the risk of infection by any of a number of potential invaders. The dose, therefore, may be somewhat higher than for therapeutic use, and success of the treatment is manifest only in a negative way, namely, in failure of infection to appear during and after the prophylactic treatment.

Once an infection is established the responsible pathogen and its sensitivity to different antibiotics can (and should) be determined in the laboratory so that the course of therapy can be properly oriented. But no such aids are available to serve as guides in selection of the proper antibiotic or its dosage for prophylaxis. The remarks in the remainder of this paper, therefore, pertain principally to prevention of wound infection. Although we stress antibiotic prophylaxis, it should be emphasized that we are considering major wounds that are deep, that cover an extensive area of the body, or that for other reasons are likely to be heavily contaminated or prone to become infected. *Routine administration of an antibiotic for every wound or minor cut is not advocated.* The risk of sensitizing the patient and of encouraging emergence of strains of microorganisms resistant to the antibiotic is too great to warrant routine use.

Under most circumstances, prudent cleansing and the natural body defense mechanisms remain efficient and safe bulwarks against infection. If, however, prophylactic use of antibiotics is deemed necessary, it should aim at suppressing all the various types of possible wound invaders. Hence, it would appear logical to employ a broad-spectrum antibiotic. However, broad-spectrum antibiotics, since they are generally administered orally, tend to

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[1] Registered brand name for chlorotetracycline assigned to the Lederle Laboratories Division of the American Cyanamid Company.

[2] Registered brand name for oxytetracycline assigned to Chas. Pfizer and Company.

alter the composition of the microflora of the digestive tract. In some instances, these changes may only result in mild but annoying gastrointestinal upset that can be controlled at least partially by concomitant administration of vitamins of the B complex. But frequently, the changes are more serious. Some of the microbial inhabitants of the intestine normally are innocuous, at least when present in relatively small numbers, but may become pathogenic when other intestinal organisms, which normally may tend to check their reproduction, are eliminated or markedly reduced in number.

The broad-spectrum antibiotics are not the only ones that may induce gastro-intestinal disturbances. Even penicillin, which can be tolerated in larger amounts than most other antibiotics, can, following oral administration, give rise to the well-known furry "penicillin tongue." That this also may be a manifestation of nutritional derangement is suggested by the fact that the tongue is restored to normal appearance promptly when traces of vitamin B_{12} are ingested or if a solution of B_{12} is sprayed over its surface.

Mixed prophylaxis generally is preferable to prophylaxis with any single narrow or broad-spectrum antibiotic because it reduces the risk of emergence of resistant strains, is more likely to control resistant organisms that might occur initially in the wound, and minimizes the risk of secondary infection being initiated by use of an antibiotic that controls most organisms but leaves the field clear for infestation by persisters or secondary invaders.

Until recently, the most dreaded contaminants of wounds were the anaerobes responsible for tetanus and gas gangrene. But with increasing use of immunizing techniques, the incidence of infections due to these organisms is diminishing.[3] Aerobic organisms such as staphylococci and streptococci which are frequent causes of septicemia are now the most common offending invaders. It is for this reason that prophylactic injection of penicillin in doses of 750,000 to 1,000,000 units daily for five days often is recommended in the event of serious wounds. Alternatively, a single

[3] If the wounded individual is known to have received immunizing treatment, administration of tetanus toxoid usually is sufficient; otherwise immune serum containing the specific antitoxin also may be indicated, although this always entails the danger of serum sickness or of an anaphylactoid reaction. To be prepared for such an emergency, there should be on hand sterile solutions of adrenalin and sterile syringes and hypodermic needles.

injection of a suitable depot penicillin—for example, 600,000 to 1,000,000 units of dibenzylethylenediamine dipenicillin (DBED)—may provide an adequate concentration of penicillin in the blood to prevent infection. In order to minimize the hazard of infection by Gram-negative organisms, streptomycin and dihydrostreptomycin (in equal divided doses totalling 1 gm. to 2 gm. daily) often are administered jointly with penicillin. Penicillin and streptomycin may be effective, either systemically or topically, in curtailing wound infection by organisms which are not among the most sensitive to them, e.g., *Proteus vulgaris* and *Pseudomonas aeruginosa*. Exposure of these organisms to concentrations of the antibiotics which do not prevent their growth *in vitro* may, none-the-less, render the organisms more susceptible to phagocytosis (Lambin, *et al.*, 1952, 1953; Oldano and Brustin, 1953).

Need for Cleansing of Wounds.—It must be emphasized that prophylactic or therapeutic use of antibiotics in wounded patients is intended to complement and not to replace other techniques of wound treatment. To inhibit or to kill a microorganism, an antibiotic must be in contact with it in a high enough concentration and for a sufficiently long time. Organisms that are ensconsed in dead tissue or tissue debris or are otherwise protected from physical contact with the drug will not be affected by it. Even in the light of newer developments and concepts, the most urgent and essential procedure is removal of foreign material that may be heavily contaminated or which, because of its physical or chemical nature, may induce inflammatory reactions. Fragments of metal if not heavily contaminated may be tolerated by the tissues for some time, and glass may remain in some tissues for years without causing noticeable effects. Wood or other organic matter, on the other hand, should be removed promptly and completely.

All dead or necrotic tissue must be removed also to prevent the biochemical chain of events causing inflammation, even under aseptic conditions, and to prevent development of infection by organisms that thrive on the degradation products of such tissue.

Biochemical changes induced even by non-infected surface wounds are not limited to the region of the trauma. Reactions following aseptic wounds depend largely on the presence of fibrinous material or other substances likely to induce fibrinolytic processes. For example, the mere implantation of a blood clot

under the skin of an animal induces an almost immediate local increase in the number of fibroplastic elements, notably of basocytes or mast cells, which soon begin to biosynthesize heparin. Within a few hours, heparin is released to effect fibrinolysis of coagulated blood, partly through an antithrombokinase effect. But enhanced activity of basocytes is not restricted to the vicinity of the clot; it becomes manifest in the connective tissue of the liver within 15 minutes after implantation (Hissard, *et al.*, 1953, Symmers, *et al.*, 1953).

If dead tissue cannot conveniently or safely be removed surgically or mechanically, chemical means may be employed. Various preparations of trypsin are available for this purpose (Miller, *et al.*, 1953a,b).

Richou and coworkers (1953) have reported that the crude fermentation products of penicillia and of actinomycetes used for industrial production of penicillin and of streptomycin, respectively, contain fibrinolytic enzymes that may be clinically useful for promoting chemical cleansing and subsequent healing of wounds. This observation may provide a partial explanation for the previously reported beneficial effects of filtrates of bacteria used jointly with sulfanilamide on infected wounds (Hattori and Kosumi, 1947).

Removal of dead or necrotic tissue has microbiologic as well as biochemical significance, since the wounded surfaces should be provided with an adequate supply of fresh lymph which is rich in the antiproteolytic factor that opposes growth of most common invaders, with the notable exception of hemolytic streptococcic and pathogenic staphylococci. These streptococcal and staphylococcal serophytes multiply freely in human blood serum and may cause septicemia unless systemic antibiotic prophylaxis is instituted.

Body defenses are adequate to cope with many infections, and care must be taken during first aid treatment not to interfere with these natural protective mechanisms. Although removal of foreign material and of necrotic tissue, which may act as a foreign body, may facilitate wound healing, more harm than good may be done if precautions are not taken against introducing organisms not originally present. Several studies have shown that microbial populations harbored by hospital staffs contain a higher percentage of antibiotic-resistant organisms than the microbial

populations at large. Therefore, if first aid is given in a hospital or by members of a hospital staff, it is doubly important to guard against superimposed infection by organisms resistant to the common antibiotics.

Irrespective of the cause and site of wound, if there is evidence of penetration deep enough so that access of topically administered antibiotics to the sites of potential infection cannot readily be effected by irrigation, instillation, or infusion, prophylactic systemic administration is indicated. Even when all portions of the wound appear to be accessible to surgical exploration and to topically applied antibiotic, systemic administration often is used to fortify the local treatment and to reduce the hazard of septicemia.

Specific Applications of Antibiotics in Wounds.—Penicillin and Streptomycin (or dihydrostreptomycin), between them, have about the same antibacterial spectrum as any one of the broad-spectrum antibiotics alone (Fig. 1). The broad-spectrum antibiotics attack also certain other classes of microorganisms, notably rickettsias, in addition to the classes of bacteria that are inhibited by penicillin and streptomycin. But this is not of major significance in emergency treatment of wounds, since the principal offending invaders in wounds are all bacteria. Therefore, generally joint use of penicillin and streptomycin is as satisfactory as use of any single broad-spectrum antibiotic for prophylactic purposes in wounds and may even be superior. These two antibiotics together usually are capable of providing as wide antibacterial protection as the broad-spectrum drugs in prophylaxis and are less likely to cause digestive disturbances and alteration of the normal microflora of the patient.

Especially in prophylaxis, where as was pointed out before the object is to inhibit growth of all potentially pathogenic invaders, mixed therapy may be desirable. Mixed therapy, whether systemic or local, should aim at associating two or more agents that act synergistically or at least additively, the object being to provide drugs that attack microorganisms via different biochemical pathways so that an organism that escapes the attack of one drug may succumb to the other. In choosing antibiotics for joint administration, care must be exercised not to introduce any component that might tend to cancel or to reduce the beneficial effect of other agents present. This subject has been discussed fully

by Pratt and Dufrenoy (1953), not only with regard to the simultaneous use of two or more antibiotics but also with respect to the joint administration of antibiotics and of other chemotherapeutic drugs.

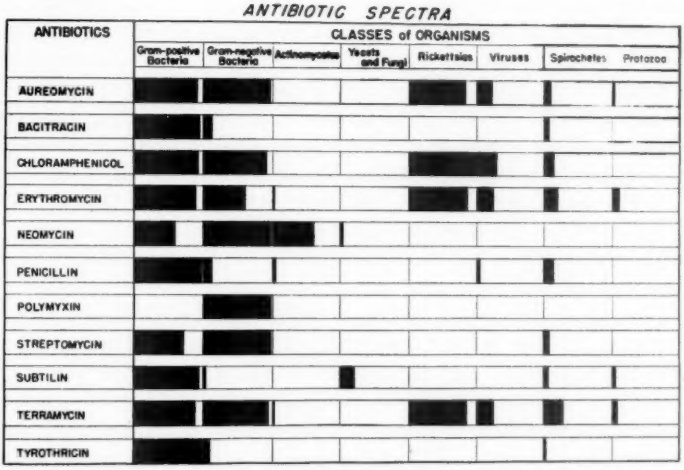

FIG. 1. Approximate *in vitro* antimicrobial spectra of 11 antibiotics. The black bands indicate the range of activity of the several drugs against different classes of microorganisms. (Reproduced from Pratt, R., and Dufrenoy, J., 1953, Antibiotics, 2nd edition, Philadelphia, Lippincott.)

The mixture of penicillin and of streptomycin or dihydrostreptomycin which has proved so effective against septicemia systemically may also be used topically, with proper adjustment of the dosage, to prevent local wound infections. However, in topical treatment one has a broader choice of antibiotics; many agents that are too toxic for systemic use may be employed safely in dressings for wounds. Indeed, there may be advantages in using such antibiotics, since by so doing sensitization of patients and development of clinically important resistant pathogens may be minimized or perhaps prevented.

The sensitizing effect of penicillin, especially when applied to the skin or mucosae, or generally in individuals with a past history of allergy, is well known. Equally well known is the tendency of organisms initially sensitive to streptomycin to give rise rapidly to progeny manifesting a high degree of resistance to

this antibiotic. These are but two reasons for the growing interest in use of other antibiotics for topical application where systemic medication is not considered necessary.

Each exposure of a microbial population to an antibiotic entails the possibility of emergence of resistant strains which may be perpetuated in human reservoirs. Therefore, from the point of view of public health it is important to avoid unnecessary exposure of actual or potential pathogens to antibiotics that are commonly used for systemic chemotherapy and, when only local or topical medication seems to be necessary, one of the antibiotics considered too toxic for systemic use, or for other reasons not so widely prescribed, may be used with advantage.

Bacitracin, *Tyrothricin*, and *Polymyxin* are finding increasing applications and may be especially suitable for treatment of certain wound conditions.

Bacitracin has about the same range of antibiotic spectrum as penicillin (Fig. 1) but often is capable of controlling resistant strains of bacteria that have emerged from parent stocks initially sensitive to penicillin. The incidence of bacitracin-resistant strains appear to be lower than the incidence of penicillin-resistant strains, perhaps because bacitracin has not been so widely abused, by indiscriminate and unwarranted use, as penicillin.

Bacitracin is suitable for use in wounds involving neurologic tissue and may prove to be the best of the currently available antibiotics for prophylaxis of such wounds or for therapy if they become infected. Bacitracin has been applied directly into and on the surface of brain tissue and other components of the central nervous system without producing untoward effects, and now in some hospitals it is considered the antibiotic of choice in neurologic infections and in compound skull fracture resulting in fungation (Teng and Meleney, 1953).

When bacitracin is administered systemically, the patient should be observed carefully for signs of proteinuria or other manifestations of toxicity. The incidence and seriousness of this problem have been markedly reduced, however, as improvements have been introduced into the method of production and purification of the antibiotic.

Bacitracin is suitable for topical use as well as systemic. Since it is biologically compatible with several other antibiotics and may even act synergistically, or at least additively, with some of

them, bacitracin often is formulated for mixed antibiotic therapy.

The increasing incidence of staphylococci and streptococci as major causes of complications following wounds, is the justification for use of ointments containing tyrothricin and bacitracin. Ointments containing 500 micrograms of tyrothricin and 500 units of bacitracin per gram have been recommended.

Bacitracin is also used in conjunction with polymyxin in ointments intended for control of both Gram-positive and Gram-negative pathogens. Such ointments generally contain 500 units of bacitracin and 10,000 units of polymyxin B per gram of ointment base. Species of *Pseudomonas* and *Proteus* are important as secondary invaders and may remain as dominant persisters following use of other antibiotics either systemically or topically. The wisdom of mixed antibiotic prophylaxis is emphasized by the observation that *Pseudomonas aeruginosa* and *Proteus vulgaris* infections often supplant skin infections due to Gram-positive organisms treated with bacitracin alone (Boger, 1953).

Following severe burns, control of *Pseudomonas aeruginosa* and other species of *Pseudomonas* often becomes a major problem. Frequently, polymyxin is the antibiotic best suited to cope with these organisms.

Terramycin has a broad antibiotic spectrum that suggests use of this drug for emergency treatment of patients acutely ill from wound infections. The antibiotic is effective against most of the organisms commonly encountered in wounds and in gas gangrene. It should be stressed, however, that only exceptional circumstances justify therapeutic use of an antibiotic without prior determination of the causal organism and its sensitivity to the antibiotic. Many organisms become so changed in morphology and in staining and other biochemical aspects following sublethal exposure to antibiotics that identification becomes extremely difficult. Therefore, if antibiotic therapy of an infection is instituted without such prior tests, a culture of the causal organism should be taken before the drug is given and should be sent to the laboratory for identification and sensitivity tests. Then if the anticipated clinical improvement is not obtained in 24 to 36 hours, another antibiotic may be chosen logically rather than haphazardly and at random.

Staphylococci highly resistant to Terramycin have repeatedly been isolated from patients following systemic Terramycin treat-

ment of pneumonia. Although a similar emergence of resistant staphylococci has not been reported following topical use of the drug, prudence suggests that it should not be used indiscriminately for prophylaxis either systemically or topically.

Terramycin deserves more study for use in wound therapy in general because of its reported ability to promote tissue growth and healing in the lesions of yaws and skin infections (Loughlin and Joseph, 1951; Wright and Tschan, 1953). However, Lund (1953), although not referring specifically to antibiotics, is of the opinion that "there is absolutely no evidence that any substance can accelerate healing beyond the optimal rate for a given wound in a given patient under ideal conditions."

Aureomycin, the broad antibiotic spectrum of which is well known, has been formulated as a dust with a methyl ester of para-oxybenzoic acid and monoacetyl urea to provide effective medication of suppurative lesions of the skin (Garnier, 1953). Such a preparation, containing 1 gm. Aureomycin, 1 gm. of the methyl ester, and 3 gm. of the urea derivative may be useful when topical application is warranted but use of wet preparations is contraindicated.

Chloromycetin,[4] like Aureomycin and Terramycin, may often be useful in prophylaxis (or in therapy) of wounds for controlling organisms that are resistant to penicillin and to streptomycin. The antibiotic can be applied topically as a dust (5 per cent in lactose) or in propylene glycol.

In view of the problems discussed above, an advantage of Chloromycetin appears to be its relatively low tendency to induce sensitization in the host or resistance among potential pathogens under clinical conditions.

Ilotycin[5] and *Magnamycin*[6] also find useful applications in treatment of wounds, especially in therapy of established infections caused by Gram-positive organisms resistant to penicillin. The tendency of Magnamycin to build up to higher concentrations in the tissues than would be expected from serum concentrations suggests special usefulness in the treatment of wounds. Since Ilotycin and Magnamycin often are less effective than peni-

[4] Registered brand name for chloramphenicol assigned to Parke, Davis & Co.

[5] Registered brand name for erythromycin assigned to the Eli Lilly Co.

[6] Registered brand name for carbomycin assigned to Chas. Pfizer and Co.

cillin against penicillin-sensitive organisms and do not have as extensive an antibacterial spectrum as the true broad spectrum drugs, it seems unlikely that they will become important in prophylactic treatment of wounds.

Special Procedures Depending on Type and Size of Wound.—From the practical point of view of antibiotic treatment, wounds may be classified as to cause, extent and depth, and site.

The most common causes of wounds until recently were mechanical injuries due to collision with solid objects, resulting in disruption of the integrity of the skin or tissues. But with development of chemical and explosives industries, wounds due to blasts and burns increased. Last but not least, short-wave radiation injuries may be considered in relation to antibiotics, not only because such radiations cause acute manifestations comparable to burns, but also because they induce insidious chronic effects, some of which may be amenable to antibiotic treatment.

Facial injuries.—Of prime importance is avoidance of any procedure that might result in unnecessary scarring. Antibiotics, by promptly controlling infection, may prevent excessive tissue destruction and its sequelae. They also facilitate restitution of normal features by plastic surgery and skin grafting.

Eye injuries.—Injury to one eye may indirectly affect the function of the other. Therefore, emergency treatment should aim not only at preserving the obviously affected eye but also at preventing any aggravation that might lead to loss of function in the other one.

In view of the extreme pain that accompanies many eye injuries, local instillation of a solution of cocaine hydrochloride often is the first emergency step. When cocaine is employed, use of sulfonamides as anti-infective agents is contra-indicated because of the anti-sulfa effect of the anaesthetic.

Even apparently superficial lesions caused by penetration of small particles into the cornea or into the eyelid call for local anaesthesia and careful removal of foreign bodies, followed by instillation of an ophthalmic Aureomycin, Terramycin or Chloromycetin preparation. Bandaging is often indicated to immobilize both eyes in order to favor healing.

In case of bursting of the eyeball, first aid is limited to local instillation of cocaine and of an ophthalmic antibiotic prepara-

tion and concomitant systemic administration of a suitable antibiotic. Bandaging is, of course, indicated.

In case of burns by chemicals, after local anaesthesia, the eye should be repeatedly washed with several liters of a tepid isotonic and appropriately buffered saline solution. Following this treatment, a suitable antibiotic preparation may be applied with an ophthalmic preparation of cortisone. Cortisone is used to prevent inflammation and excessive scar formation which might impair vision.

Hand and Foot Injuries.—Preservation of function of the extremities depends principally on preservation and, if needed, restoration of the tendons. Judicious use of antibiotics may ensure success of modern surgical procedures that promptly restore functional integrity and prevent crippling immobilization (Mason, 1953, Lorthioir, 1953). A major responsibility in the first treatment of such injuries, in addition to attempting to prevent generalized infections, is to ensure prompt procurement of proper surgical care.

Burns.—Symptoms commonly described as burns may arise from three main causes, namely, chemicals, heat, and short-wave radiations. The acute manifestations of injury caused by any of these destructive agents may be similar in gross aspects or they may differ. Short-wave radiations, such as X-rays or radiations from atomic blasts, are the most insidious since they are capable of producing far-reaching chronic effects.

The first step in emergency treatment of chemical burns is to remove the offending agent from exposed areas of the body by copious washing and to remove contaminated clothing. If the specific substance responsible for a chemical burn is known, primary washing may be followed by rinsing with neutralizing agents. Once the offending chemical has been removed so far as possible, emergency measures for treatment of burns are essentially similar whether the cause is chemical or physical. If the cutaneous protection of the body has been destroyed over a large area or in the case of penetrating burns, prophylactic use of antibiotics may be indicated. Generally, in such instances, both systemic and topical administration are indicated. Appropriate early use of antibiotics in burns, by eliminating pathogens, especially streptococci, enhances the chances of success and minimal scarring when skin grafting is necessary.

Terramycin, which has been unusually successful in the treatment of afflictions of the skin, likewise has had wide acceptance in prophylactic and therapeutic treatment of burns.

After-effects of Radiation Injury.—Among the delayed or chronic effects that may result from excessive exposure to radiations of short wave lengths are decreased resistance to infection (possibly due to destruction of leukocytes and other components of blood), anemia, and sterility. Although these effects may seem to lie beyond the range of emergency treatment, some of them cannot be ignored in discussion of emergency use of antibiotics in wounds because they may profoundly influence the ultimate fate of the patient.

There is accumulating evidence that intravenous injection of appropriately treated plasma proteins or extracts of spleen or other organs may complement the action of antibiotics in reducing the hazard of infection incident to excessive but sublethal irradiation (Ardry, *et al.*, 1953; Marston, *et al.*, 1953.)

Shock.—Whether resulting from trauma or burns, shock is a common serious consequence of injury and requires emergency treatment. After all, the first concern is to keep the patient alive until the natural body defense mechanisms, perhaps fortified by antibiotic therapy, can rehabilitate the victim.

Normally, blood or plasma would be used to counteract shock; but in case of a major disaster, supplies of blood and plasma probably would be soon exhausted, and recourse to substitute blood expanders would become necessary.

Antibiotics administered orally are poorly absorbed when the patient is in hemorrhagic or traumatic shock. It is fortunate, therefore, that some of the plasma substitutes may be used as vehicles for systemic antibiotics. For example, polyvinylpyrrolidone has been used routinely when prolonged effect of penicillin has been desired (Janot, 1950). In the absence of specific substitutes, sterile saline may be used in treatment of shock (Rosenthal, 1953), and this also is a suitable emergency vehicle in which to administer antibiotics.

Summary. Leukocytes are the best guardians of wounds. Natural protective mechanisms are able to prevent infection and to cope with minor infections in many wounds.

Antibiotics should not be administered routinely for prophylaxis or therapy of all wounds, but should be reserved for special

use. Even when conditions warrant use of antibiotics prophylactically or therapeutically in wounds or burns, the drugs should be considered only to complement—and not to replace— other techniques of treatment.

Antibiotics cannot act by remote control: to be effective, they must be in actual contact with the pathogens for a sufficient length of time to effect inhibition. Therefore, cleansing of wounds is important.

If antibiotic prophylaxis is instituted, it is necessary to attempt to control all potentially pathogenic invaders. For this purpose joint use of two antibiotics that attack organisms via different biochemical pathways is preferable to use of a single antibiotic.

When topical application alone is adequate, it is desirable to employ antibiotics not generally used for systemic medication. This is to avoid the risk of inducing unnecessary sensitization of the patient to the common antibiotics and to minimize the hazard of inducing emergence of strains of organisms resistant to these antibiotics.

For penetrating wounds or for wounds that cannot be cleansed properly, if topical antibiotic preparations are employed, they should be supported by systemic antibiotics also.

Selection of the appropriate antibiotic for therapy of an established infection is best based on prior identification and determination of the sensitivity of the pathogen.

Sublethal exposure of bacteria to an antibiotic may so alter their morphology and biochemical behavior that subsequent identification is extremely difficult and uncertain. Therefore, in the rare instances when emergency conditions seem to dictate immediate institution of antibiotic therapy, a culture should be taken at the same time and sent to the laboratory for identity and sensitivity tests so that if clinical improvement is not manifest in 24 to 36 hours, another antibiotic may be chosen logically rather than at random.

Specific recommendations for use of the different antibiotics and for concomitant and supportive procedures are made.

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NOTE: *The references listed below have been selected as authoritatively representative of recent developments in their respective fields. All are recommended for reading, but not all of them are cited specifically in the text.*

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PROCEEDINGS, TEXAS BRANCH OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

MAY 29-30, 1953

Roy and Lillie Cullen Building,
Baylor University College of Medicine,
Texas Medical Center, Houston, Texas

PAPERS

1. A comparison of the Fungistatic Properties of Three Aromatic Diamidines. Willson J. Fahlberg, Department of Microbiology, Baylor University College of Medicine, Houston.

2. Further Studies of Erythromycin Activity Against Corynebacterium Diphtheriae. Robert G. Brown and Frank B. Engley, Jr., Department of Bacteriology and Parasitology, The University of Texas Medical Branch, Galveston.

3. Effect of Neomycin on the Intestinal Flora. John H. Davis, Jr., Anthony A. Contreras, and Rudolph Massari, Surgical Research Unit, Brooke Army Hospital, Fort Sam Houston, San Antonio.

4. Inhibition of Blood Antibacterial Action by Gastric Mucin. Frank B. Engley, Jr., Martha Jordan, and Carl F. Page, Department of Bacteriology and Parasitology, The University of Texas Medical Branch, Galveston.

5. Stages in the Life Cycle of Litomosoides Carinii. (Motion picture.) Etta Mae Macdonald and J. A. Scott, Department of Preventive Medicine and Public Health, The University of Texas Medical Branch, Galveston.

6. Bacteriophage Typing of Staphylococci Isolated in a Burns Treatment Ward. Russell E. Brame, Ruth L. Latta, and L. R. Kuhn, Surgical Research Unit, Brooke Army Hospital, Fort Sam Houston, San Antonio.

7. Ratio of Colored and Colorless Variants of Serratia Marcescens Before and After Ultraviolet Irradiation. R. P. Williams and R. Miller, Department of Microbiology, Baylor University College of Medicine, Houston.

8. An *In Vitro* Technique for Determining pH Changes in Dental Plaque in the Presence of Saliva. W. A. Nolte and S. S. Arnim, The University of Texas Dental Branch, Houston.

9. A Simple Medium for Counting Clostridium Spores. William R. Schmieding and E. Staten Wynne, Division of Experimental Pathology, The University of Texas M. D. Anderson Hospital for Cancer Research, Houston.

I. M. LEWIS LECTURE

Dr. M. R. J. Salton
Microbiological Chemistry Unit, School of Biochemistry
Cambridge University, England

"The Preparation and Chemical Constitution of Bacterial Cell Walls."

10. *In Vitro* Testing for Leptospiracidal Drugs. L. C. Kuhnley and V. T. Schuhardt, Pathological Microbiology Laboratory, Clayton Foundation; and Department of Bacteriology, The University of Texas, Austin.

11. Attempts to Prepare Leptospiral Complement Fixing Antigens. Robert M. Pike, Henry B. Owens, and Robin I. Burdette, Department of Bacteriology, Southwestern Medical School, The University of Texas, Dallas.

12. The Use of a Blood Medium for Routine Isolation of M. Tuberculosis. Virginia S. Crane, Albert M. Richmond, and Donald H. Hunter, 4th Army Area Medical Laboratory, Fort Sam Houston, San Antonio.

13. The Microbiological Approach to Research in Cancer Diagnosis. Cora L. Gott and E. Staten Wynne, Division of Experimental Pathology, The University of Texas M. D. Anderson Hospital for Cancer Research, Houston.

14. A Cyto-Immunological Response to Carcinogenesis. M. Pollard and R. H. Bussell, Department of Preventive Medicine and Public Health, The University of Texas Medical Branch, Galveston.

15. Effect of Cortisone on Experimental Rickettsioses and on Antibody Formation. Dorothy M. Whitney and Ludwik Anigstein, Rickettsial Research Laboratory, The University of Texas Medical Branch, Galveston.

16. Observations on Immunity in Psittacosis. J. V. Irons, M. L. Denley, and Thelma D. Sullivan, Bureau of Laboratories and Division of Epidemiology, State Health Department, Austin.

17. Mixed Infections with Coxsackie and Lansing Poliomyelitis Viruses in Mice. S. Edward Sulkin and Craig Wallis, Department of Bacteriology, Southwestern Medical School, The University of Texas, Dallas.

18. Heterophile Antibody Response to Immunization with Influenza Virus. V. T. Schuhardt, Marylyn M. Willsie, and Thomas D. Sullivan, Pathological Microbiology Laboratory, Clayton Foundation; Department of Bacteriology, The University of Texas; and State Department of Health Laboratory, Austin.

19. *In Vitro* Testing of Brucellacidal Antibodies Produced in Rabbits Immunized with Brucella Abortus Antigens. Bob A. Freeman and V. T. Schuhardt, Brucellosis Laboratory, Clayton Foundation; and Department of Bacteriology, The University of Texas, Austin.

Effect of Neomycin on the Intestinal Flora. John H. Davis, Jr., Capt., MC, Anthony A. Contreras, and Rudolph J. Massari. Surgical Research Unit, Brooke Army Medical Center.

Studies on the suppressive action of Neomycin Sulfate on the intestinal flora have been performed. Volunteer laboratory personnel and patients of this hospital have shown, with a regimen of a liquid diet, phosphate of soda, and Neomycin, a marked reduction in the intestinal flora. Neomycin Sulfate has proved effective for inhibiting both gram positive and gram negative bacterial organisms found within the intestine.

Bacteriophage Typing of Staphylocicci Isolated in a Burns Treatment Ward. Russell E. Brame, Ruth L. Latta, and L. R. Kuhn, Lt. Col., MSC, Surgical Research Unit, Brooke Army Medical Center.

Coagulase-positive staphylococci may be typed by testing for patterns of susceptibility to standard staphylococcal bacteriophages. The methods are described and typical patterns illustrated. Typing of pathogenic staphylococci from the lesions of patients in a burns treatment ward reveal the existence of single and multiple infections with relatively few types predominating. Phage-typing of staphylococci isolated from the environment of patients indicate possible sources of infection.

A Simple Medium for Counting *Clostridium* Spores. William R. Schmieding and E. Staten Wynne. Division of Experimental Pathology, The University of Texas M. D. Anderson Hospital for Cancer Research, Houston, Texas.

An easily prepared medium has been devised as a substitute for the complex modified Yesair's pork infusion agar of Wynne and Foster (J. Bact. 55: 61–68, 1948) for counting *Clostridium* spores. Buffered yeast extract agar containing BBL thioglycollate supplement, 0.1% starch, and 0.1% sodium bicarbonate yielded spore counts with five *Clostridium* strains which were at least equal to those obtained in modified Yesair's agar, even when the latter medium was supplemented with sodium bicarbonate. Added bicarbonate resulted in a statistically significant increase in final counts with *C. botulinum* 62A in modified Yesair's agar, and with *C. botulinum* 115B in the yeast extract medium. Futher-

more, bicarbonate added to Yesair's agar permitted earlier colony development with *C. botulinum* 62A and allowed counts to be made at 24-48 hours.

The Microbiological Approach to Research in Cancer Diagnosis. Cora L. Gott and E. Staten Wynne. Division of Experimental Pathology, The University of Texas M. D. Anderson Hospital for Cancer Research, Houston.

Excluding etiological investigations, microbiological approaches to research in cancer diagnosis have apparently not been reported. Microorganisms are capable of sensitive responses to variations in known and possibly unknown components in blood, and a single organism may be able to respond to changes in more than one component, whether these changes occur singly or simultaneously.

The feasibility of the microbiological approach was illustrated by growth responses of 4 strains of lactic acid bacteria to tungstic acid filtrates of serum in a yeast extract medium. Results were given for 36 individuals with malignant neoplastic conditions, 7 with benign neoplastic conditions, 7 with hyperplasias, 6 with non-neoplastic diseases, and 35 apparently healthy individuals. Combined growth responses of the 4 organisms gave higher values with normal sera than with sera from cancer patients, with little overlapping. However, the other 3 groups gave results tending to simulate those with cancer patients. Youden's index was 0.746 for the malignant neoplastic and normal groups only, but 0.484 with all 5 groups. This test procedure is regarded as of no practical diagnostic value, but it is considered to indicate a potential usefulness of the microbiological type of approach in this field.

In Vitro Testing of Brucellacidal Antibodies Produced in Rabbits Immunized with Brucella Abortus Antigens. Bob A. Freeman and V. T. Schuhardt, Brucellosis Laboratory, Clayton Foundation; and Department of Bacteriology, The University of Texas, Austin.

In the brucellacidal antibody test described the antiserum, the complement and the live *Brucella abortus* antigen (100-300 cells/0.1 ml.) were pipetted aseptically to sterile ¾ x 7 inch

tubes. After 4 hours incubation at 37 C melted tryptose agar at 45–50 C was added to the tube, which immediately was rotated in a spinning device immersed in a cold bath until the agar solidified on the walls of the tube. Colony counts of test and control preparations were made after 4 to 6 days incubation at 37 C. Results are expressed as the percentage of the inoculated organisms killed in the test system. The regular occurrence of a Neisser-Wechsberg antibody excess zone of no killing in high serum concentrations when the sera show moderate to high agglutinin titers tends to convince us that *Brucella* agglutinins probably serve to protect *B. abortus in vivo* against the killing effect of brucellacidal antibodies distinct from the agglutinins remains to be determined.

Ratio of Colored and Colorless Variants of *Serratia marcescens* Before and After Ultraviolet Irradiation. Robert P. Williams and Reba Miller, Department of Microbiology, Baylor University College of Medicine, Houston.

Serratia marcescens, Baylor, yields no stable white variant colonies when surface plated on Bunting's synthetic medium. If a suspension of the organism is treated with ultraviolet light of such intensity as to kill 99% of the cells, white variants appear. In a population of 2.9×10^5 ultraviolet irradiated survivors, three per cent were stable white variants.

A red strain isolated from the parent Baylor strain after ultraviolet treatment behaved as the non-ultraviolet treated strain. No stable white variants appeared in cell suspensions not treated with ultraviolet light. After exposure to ultraviolet irradiation white variants appeared. In a population of 4.9×10^5 survivors, one and one-half per cent were stable white variants.

A white variant isolated after treating the parent strain with ultraviolet light proved to be extremely stable. No red variants appeared either before or after exposure to ultraviolet irradiation.

Effect of Cortisone on Experimental Rickettsioses and on Antibody Formation. Dorothy M. Whitney and Ludwik Anigstein, Rickettsial Research Laboratory, The University of Texas Medical Branch, Galveston, Texas.

The purpose of this study is to evaluate the effect of cortisone on experimental spotted fever and epidemic typhus as to the clinical course, antibody levels and immune responses. Cortisone was injected into guinea pigs (25 mg. daily for 8 to 12 days) during immunization against spotted fever with the specific vaccine. Complement fixation titers of the cortisone-treated series were, on the average 1:8 as compared with 1:13 of the non-treated group. Both groups showed solid immunity after reinoculation with spotted fever. Cortisone treatment of guinea pigs infected with epidemic typhus resulted in shortening of incubation and prolongation of fever. Rabbits treated with cortisone during vaccination against typhus showed a lowering of complement fixation titers (average 1:50) as compared with the non-treated controls (1:93). Cortisone administration to guinea pigs and rabbits during immunization with rickettsial antigens lowers the level of the specific antibody. The suppressive action is of low degree only.

Yeast Extract Starch Bicarbonate Agar for Recovery of Drastically Heated *Clostridium* Spores. E. Staten Wynne, William R. Schmieding, and George Day. Department of Pathology (Division of Experimental Pathology), The University of Texas, M. D. Anderson Hospital for Cancer Research, Houston, Texas.

For counts of mildly heated spores, it was reported previously that a simple yeast extract starch bicarbonate medium (YESB) yielded results comparable to those obtained with the modified Yesairs' pork infusion starch agar routinely used in this and other laboratories. The present study concerns a comparison of these two media for recovery counts of *drastically* heated spores.

Organisms used were *Clostridium botulinum* 62A, *Clostridium chauvei*, *Clostridium histolyticum*, *Clostridium perfringens*, and putrefactive anaerobe No. 3679. The spores were heated in boiling water for periods sufficient to insure destruction in excess of 99.95 per cent for all species. Recovery counts in the two media were not significantly different.

On the basis of the limited data presented, it is of course not possible to conclude that YESB agar should be adopted as the recovery medium for heat resistance and related studies. However,

it is felt that these findings suggest more exhaustive studies by other investigators more directly concerned with recovery of severely heated spores. Should the values of YESB as a recovery medium be confirmed, its use in place of modified Yesairs' or other infusion media might effect a not inconsiderable saving of time and effort.

PROCEEDINGS, TEXAS BRANCH OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

Fall Meeting

Texas Branch of the Society of American Bacteriologists
Auditorium of the Texas Medical Association Library, Austin

1. A preliminary trial of the polyphosphate method of Jacobs and Gillis for the purification of diphtheria toxoid. M. K. Dulaney and L. Gerhardt, Biologics Division, Texas State Department of Health, Austin.

2. Preliminary results on the preservation of vaccinia virus by freeze-drying with various stabilizers. P. J. Forsyth and E. B. M. Cook, Biologics Division, Texas State Department of Health, Austin.

3. Population changes in gonococcus cultures with reference to colonial types. Mrs. Alice Conklin and C. E. Lankford, Department of Bacteriology, The University of Texas, Austin.

4. A comparative study of antibiotic sensitivity tests with an evaluation in treated patients. Sol Haberman, Baylor University Hospital, Dallas.

5. Use of hemoglobin indicator for rapid method of determining antibiotic sensitivity of micro-organisms. J. L. W. Jackson, W. E. Dye, and R. B. Mitchell, Department of Microbiology, USAF School of Aviation Medicine, Randolph Field.

6. Agar diffusion method for estimating antimicrobial activity of combinations of chemotherapeutic agents. W. E. Dye, R. B. Mitchell, and J. L. W. Jackson, Department of Microbiology, USAF School of Aviation Medicine, Randolph Field.

7. Infrared absorption studies on virus preparations. Albert A. Benedict, Virus Research Laboratory, The University of Texas Medical Branch, Galveston.

8. Viral oncolysis with human tumors. Morris Pollard and Clifford D. Snyder, Virus Research Laboratory, and Department of Surgery, The University of Texas Medical Branch, Galveston.

9. Growth in sub-bacteriostatic concentrations of streptomycin and the emergency of resistance. Eugene D. Rosenblum, Department of Microbiology, Southwestern Medical School of The University of Texas, Dallas.

10. Alteration of bacterial flora of the bowel following intestinal antisepsis. Edgar J. Poth and Maurice Adams, Surgical Research Laboratory, The University of Texas Medical Branch, Galveston.

11. The use of K-agar for the growth of organisms used in the production of bacterial vaccines. J. Field, R. A. Grote, and E. B. M. Cook, Biologics Division, Texas State Department of Health, Austin.

12. The disposal of radioiodinated antirickettsial homologous gamma globulin in control and specifically immunized rabbits. Ludwik Anigstein, Dorothy Whitney, and William Barnes, Rickettsial Research Laboratory, The University of Texas, Galveston.

13. Influence of antigen impurities upon adsorption-agglutination of tannic acid treated red cells. Paul Donaldson, Department of Microbiology, Southwestern Medical School of The University of Texas, Dallas.

14. Current studies on anthrax in Texas. Kenneth L. Burdon and Reuben D. Wende, Department of Microbiology, Baylor University College of Medicine, and Houston City Health Department Laboratory, Houston.

15. The nucleic acid content of virulent and avirulent anthrax organisms. E. O. Bennett and R. P. Williams, Department of Biology, University of Houston, and Department of Microbiology, Baylor University College of Medicine, Houston.

16. Notes on the Sixth International Congress of Microbiology held at Rome, September 6–12, 1953. Frank B. Engley, Department of Bacteriology, The University of Texas Medical Branch, Galveston.

17. Oxygen Tension as a possible factor in the virulence of the tubercle bacillus. L. Ruth Guy, Sidney Raffel, and C. E. Clifton, Parkland Hospital, and Southwestern Medical School of The University of Texas, Dallas, and Stanford University, San Francisco.

18. The growth of pleuropneumonia-like organisms of human origin. Robert Keller* and Harry E. Morton, Department of Microbiology and the Institute for Study of Venereal Diseases, University of Pennsylvania, Philadelphia. (* Present address: The University of Texas M. D. Anderson Hospital for Cancer Research, Houston.)

19. Yeast extract starch bicarbonate agar in recovery of drastically heated *Clostridium* spores. E. Staten Wynne, William R. Schmieding, and George Day, The University of Texas M. D. Anderson Hospital for Cancer Research, Houston.

20. The proteolytic action of *Streptococcus lactis in milk*. W. Carl van der Zant, Texas A. & M. College, College Station.

21. Observations on the inhibition of certain fungi by testosterone propionate. Enrique Leal, Jack R. Whitaker, and Robert L. Ingersoll, the Laboratory Service, Brooke Army Hospital, Brooke Army Medical Center, Fort Sam Houston.

The Nucleic Acid Content of Virulent and Avirulent *Bacillus anthracis*. E. O. Bennett, Jr. and Robert P. Williams, Department of Biology, University of Houston and Department of Microbiology, Baylor University College of Medicine, Houston, Texas.

Twenty cultures of *Bacillus arthracis*, equally divided among virulent and avirulent strains, were analyzed by the Schmidt

and Thannhauser method for their nucleic acid content. The results showed that the total nucleic acid content and the pentosenucleic acid content were very similar for virulent and avirulent strains. The desoxypentosenucleic acid fraction of virulent strains was about half again as large as that of avirulent strains. The average DNA content, expressed as milligrams per cent of phosphorus per milligram of dry weight of cells, was 0.81 mg. for virulent organisms and 0.55 mg. for avirulent. In no case was the desoxypentosenucleic acid fraction of virulent organisms as low as the greatest amount of the acid in any avirulent organism. The ratio of pentosenucleic acid to desoxypentosenucleic acid was generally lower in virulent strains than in avirulent.

The significance of these results is not known. We are extending our studies to more anthrax strains and we hope to analyze the nucleic acid fractions qualitatively as well as quantitatively.

Proteolytic activity of *Streptococcus lactis* in milk. W. C. van der Zant, Dairy Husbandry Department, Texas A. & M. College, College Station, Texas.

Protein-free fractions prepared from milk after incubation with *Streptococcus lactis* for different lengths of time were investigated for their content of amino acids and peptides by paper chromatography. Evidence was presented for the presence in the protein-free fraction of three peptides at 0 hr., five peptides after 24 hrs., and six peptides after 96 hrs. of incubation.

Use of Hemoglobin Indicator for Rapid Method of Determining Antibiotic Sensitivity of Microorganisms. J. L. W. Jackson, William E. Dye, and Roland B. Mitchell. Department of Microbiology, USAF School of Aviation Medicine, Randolph Air Force Base, Texas.

This paper presents a modification of a standard procedure for the determination of the sensitivity of microorganisms to antibiotics which will markedly reduce the time of incubation required before the tests can be read.

Suspensions of the organisms are seeded in melted cooled brain heart infusion agar plus 0.8 per cent dextrose and poured into petri dishes containing thin layers of heart infusion agar plus 20 per cent citrated whole human blood. Filter paper disks impreg-

nated with various antibiotics are placed on the surface of the medium and the tests incubated in the usual manner. Prior to the appearance of visible growth, the metabolism of most strains of microorganisms will develop relatively intense reducing conditions within the medium which are reflected by reduction of the hemoglobin and characteristic change in color of the blood in the base. Zones of inhibition of hemoglobin reduction surrounding the antibiotic containing disks are similar in size to those measured from inhibition of visible growth produced after further incubation of the plates or on controls inoculated by the conventional surface-streaking procedure.

The incubation period required for sufficient hemoglobin reduction or visible growth to clearly discern zones of inhibition around the antibiotic-containing disks varies with the nature of the test organism and the size of the inoculum employed. In general, the zones of inhibition on any given plate can be measured by the hemoglobin reduction method in about one-half of the time required for visible growth to appear. Tests with heavy inocula (up to 1–100) of the bacteria commonly encountered in the clinical laboratory can usually be read in two hours by the hemoglobin reduction method.

Agar Diffusion Method for Estimating Antimicrobial Activity of Combinations of Chemotherapeutic Agents. William E. Dye, Roland B. Mitchell, and J. L. W. Jackson. Department of Microbiology, USAF School of Aviation Medicine, Randolph Air Force Base, Texas.

An agar diffusion method has been developed for studying the bacteriostatic action of combinations of antimicrobial agents. This technique is based on diffusion of the drugs from submerged filter paper strips and measurement of the pattern of growth inhibition in the area where the drugs interdiffuse. Six different types of combined drug effects have been demonstrated and a rational interpretation suggested for each.

Observations on the Inhibition of Certain Fungi by Testosterone Propionate. Enrique Leal, Jack R. Whitaker and Robert L. Ingersoll, the Laboratory Service, Brooke Army Hospital, Brooke Army Medical Center, Ft. Sam Houston, Texas.

The fungistatic activity of the diamidines *in vitro* was noted by Elson, W. O. (J. Infect. Dis. 76: 193, 1945). There is a chemical similarity of stilbamidine, an aromatic diamidine, to diethylstilbesterol ,Curtis, A. C., Harrell, E. R., Arch. Derm. 66: 676–690, 1952).

When cultures of primary isolates of various fungi were cultured on Sabauraud's dextrose agar containing calculated amounts of testosterone there is definite inhibition of certain of the test organisms. Testosterone propionate showed a definite fungistatic effect against *Trichophyton rubrum*, mentagrophytes and tonsurans at a minimal inhibitory concentration of 0.1 mg. per milliliter.

Stilbamidine which appears as a specific drug for blastomycosis was used in the same concentrations for comparison. Stilbamidine demonstrated its usual antifungal activity against *Blastomyces dermatitidis* and had an inhibitory effect on *Coccidioides immitis* which decreased its m.i.c. approximately ten times from that of testosterone.

Testosterone propionate appears to have some fungistatic effect upon the Trichophytons.

The possible antimycotic action of other steroides is being studied. More complete investigations of the steroides may develop an antimycotic agent of therapeutic value.

ELECTRON MICROSCOPIC STUDIES OF A VIRUS ISOLATED FROM A PATIENT WITH A COMMON COLD*

REGINALD L. REAGAN,[1] EDDY D. PALMER,[2] MILDRED T. STEWART,[3] AND ARTHUR L. BRUECKNER[4]

The common cold (acute coryza, acute rhinitis) is attributable to infection of the upper respiratory tract with a virus. The symptoms and signs indicate the presence of an acute but transient alteration in the physiology of the mucous membrane of the upper respiratory tract, particularly that lining the nose and the paranasal sinuses. (Horsfall) Dochez *et al.* (1930) first reported successful transmission of the common cold to chimpanzees by means of filtrates (Dochez, Shebley and Mills, 1930). Recent studies by Andrews (1949) with the collodion membrane of graded porosity indicate that the virus may have dimensions of 40 to 50 mu or less. Andrew's (1950) results of tests for immunity to reinfection suggest that resistance may develop and persist for three or four months.

Materials and Methods. Nasal and throat saline washings were obtained two days after the onset of a common cold from Roger Milstrey (25 years old) a graduate student at the University of Maryland. The clinical picture was typical naso-pharyngitis with a slight soreness or roughness of the throat. The nasal and nasopharyngeal mucosa was swollen and injected, and a headache was present. The patient's temperature did not rise above 100° F. For the normal control saline washings were obtained from the nose and throat of a student having no history of a cold for the past year. This student was in excellent health.

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* From the Virus Laboratory, Live Stock Sanitary Service, University of Maryland, College Park, Maryland, and the Gastrointestinal Section, Walter Reed Army Hospital, Washington, D. C. Submitted for publication September 18, 1953.

We thank Dr. Harry Bishop and Dr. Royal Reynolds from the Unive sity of Maryland Infirmary for confirming the diagnosis of the common cold in the patient.

[1] Professor of Virus Diseases.

[2] Chief, Gastrointestinal Section, Walter Reed Army Hospital, Lieutenant Colonel, Medical Corps, U. S. Army and Consultant in Gastroenterology to the Surgeon General.

[3] Virologist.

[4] Director, Live Stock Sanitary Service and Professor.

Two young chimpanzees weighing from 10 to 12 pounds each were used throughout the experiment. One chimpanzee which was received previously had a cold upon arrival. This chimpanzee was put in isolation for a period of three weeks after recovery from the cold. The second chimpanzee was also put in isolation before exposure to the virus. During this isolation period the two chimpanzees showed no evidence of having any respiratory symptoms. Before exposure to the isolated virus both chimpanzees appeared normal. The animals were carefully examined each day for respiratory symptoms. Rectal temperature was not taken. A diet consisting of milk, bananas, oranges, bread, potatoes, greens and chimp crackers was provided throughout the experiment.

Experimental. The infected nasal and throat washings from Roger Milstrey and the normal nasal and throat washings from the second student were each filtered through a Type St Size L3 Seitz filter. The filtrates were then subjected to centrifugation under refrigeration in a Spinco ultracentrifuge for two hours at 44,770 R.P.M. The temperature of the refrigerated outer jacket stayed constant at –8°C during the centrifugation. After the two hour centrifugation the supernatants from the specimens were discarded, and the sediments from each specimen were re-suspended in 1.0 cc. of chilled physiological saline. Several drops of each specimen were placed on several parlodion film supports which had been prepared 92 hours previously. After all excess fluid had been removed with small capillary pipettes the films were dried and shadowed with chromium (William and Wyckoff, 1946) at arc tangent 4/12 and examined under the R.C.A. electron microscope, type E.M.U. The balance of the material of infected washings was injected intranasally into chimpanzee No. 1 (immune to cold virus) and into chimpanzee No. 2 which was not immune. On the third day the chimpanzee No. 2 showed respiratory symptoms such as coryza with a slight cough. These symptoms lasted for six days. In chimpanzee No. 1 there was no evidence of respiratory symptoms from the virus isolated from a patient with a common cold over a 10 day observation period.

Upon electron microscopic examination of the concentrated suspension from the infected nasal and throat washings uniform virus-like particles were observed. They were present in 50 per cent of the 1,000 microscopic grid fields examined.

Figures 1 and 2 are electron micrographs showing these virus-like particles. These particles are spherical in shape and measure 80 to 90 mu by direct measurement. These bodies resemble the

virus of influenza described by Stanley (1946) and the virus of measles described by Reagan *et al.* (1952).

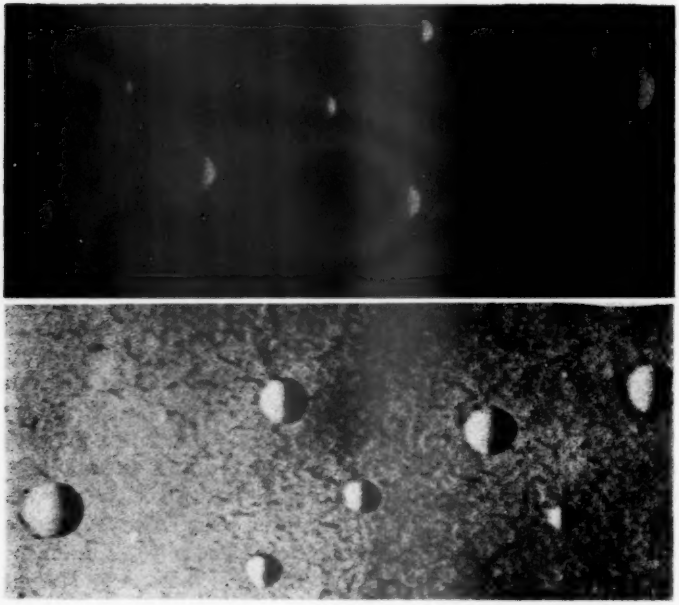

FIG. 1. Electron Micrographs of a Virus Isolated from a Patient with a Common Cold. Shadowed with Chromium at Tangent 4/12. x 60,000.

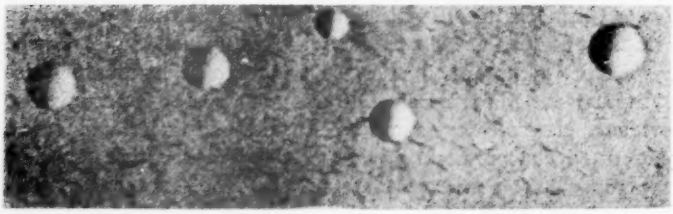

FIG. 2. Electron Micrographs of a Virus Isolated from a Patient with a Common Cold. Shadowed with Chromium at Tangent 4/12. x 90,000.

No virus-like particles were seen in the concentrated normal nose and throat washings in approximately 1,000 fields examined by electron microscopy.

Summary. Studies by electron microscopy of infected nasal and throat filtered washings fron an adult effected with a common cold showed the virus isolated to be spherical. The virus particles have a diameter of 80 to 90 mu by direct measurement. These bodies could not be demonstrated in normal nose and throat filtrate washings subject to the same procedure of concentration and electron microscope examination. The virus in the infected nasal and throat washings was infectious for the one susceptible chimpanzee but had no effect on the other chimpanzee (which previously had common cold signs and symptoms and had recovered) which was presumed to be immune.

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ELECTRON MICROSCOPIC STUDIES OF ERYTHROCYTES FROM A PATIENT AFFECTED WITH HODGKIN'S DISEASE*

REGINALD L. REAGAN, EDDY D. PALMER, EDWARD C. DELAHA, MILDRED T. STEWART, AND A. L. BRUECKNER

Hodgkin's disease (Lymphadenoma) is a chronic advanced fatal disease of unknown etiology, manifested by painless and progressive enlargement of the lymph nodes and frequently of the spleen and liver. In the later stages, fever, anemia and cachexia are prominent. Many investigators consider it a malignant neoplastic entity; others an infectious granuloma. Various organisms have been suspected such as the tubercle bacillus, the Brucella organism and a filterable virus. Hodgkin's disease is more prevalent among males than females, in the ratio of 7:3. It may occur at any age, but its greated incidence is in the 3rd and 4th decades of life. The disease has little racial predilection. (1) The disease is a hyperplastic process which involves the reticuloendothelial system and tends to become generalized. The lymphnodes, spleen, liver and bone marrow are chiefly involved.

Clinical Abstract: A 30-year old soldier, whose past medical history was not significant, was hospitalized because of two months of feverish feeling, night sweats and an enlarging neck. Examination showed low-grade fever and striking lymphadenopathy of the anterior node chains. The lacrimal gland on the left was moderately enlarged. The white blood count was 20,000, with 92% lymphocytes. The sedimentation rate was elevated. Chest plate showed bilateral hilar lymphadenopathy. The admission cervical node biopsy revealed Hodgkin's disease.

Nitrogen mustard therapy, 37 mgm. over four days, produced almost complete resolution of the cervical nodes. A month later, however, both the cervical and the mediastinal nodes were larger, leading to radiation therapy, 2500 r (skin dose) to each cervical chain and 1500 r to the mediastinum. Response was good, with disappearance of toxic signs and symptoms. Blood was drawn for virus studies four and a half months following nitrogen mustard therapy and two months after the completion of radiation. The patient was at the time in remission.

Materials and Methods. Five cc. of venous blood was collected from the above patient and placed in sterile 50 cc. erlenmeyer flasks which contained

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7 mg. of heparin in 6 cc. of sterile physiological saline. Ten cc. of venous blood was drawn likewise from a patient with no history of having had this disease (control). The flasks of infected heparinized blood and the flask of normal heparinized blood were then placed in the —4° C. ice box for one hour. They were then removed from the ice box and allowed to stand at room temperature for ten minutes with occasional rotation of each flask. Several drops of each suspension were placed on several parlodion film supports which had been prepared 24 hours previously. After all excess fluid had been removed with small capillary pipettes, the films were then washed with sterile distilled water, dried and shadowed with chromium (Williams and Wyckoff, 1946)[2] at arc tangent 4/14 and examined under the R.C.A. electron microscope, Type E.M.U.

Results

Upon examination of the specimen under the electron microscope, no virus-like particles were observed in the erythrocytes

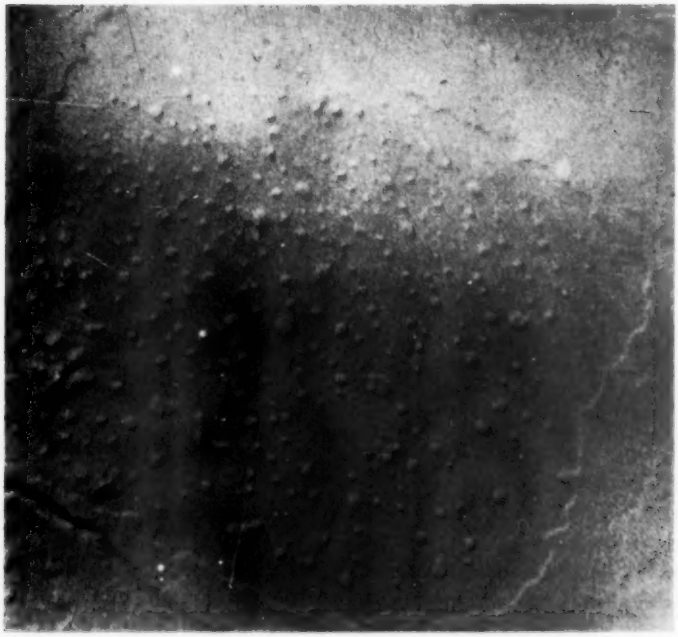

FIG. 1. Electron micrograph of an erythrocyte from a patient affected with Hodgkin's disease. Shadowed with chromium at arc tangent 4/14. Erythrocyte loaded with virus-like particles. x 40,000.

from the normal control group in approximately 2,000 fields examined, Figure 1 and Figure 2 are electron micrographs showing these virus-like particles on the surface of and in the erythrocytes. The majority of these particles are spherical in shape and measure 110 to 120 mu by direct measurement. Irregular con-

FIG. 2. Electron micrograph of a particle of an erythrocyte from a patient affected with Hodgkin's disease. Shadowed with chromium at arc tangent 4/14. This is the same erythrocyte shown in Fig. 1 enlarged to show structure of virus-like particles. Some are indicated by arrows. x 60,000.

tours may be seen on some particles. Erythrocytes from the infected blood may be found containing no virus-like particles. Figure 3 shows an erythrocyte from the normal blood containing no virus-like particles.

Summary. Studies by electron micrographs of erythrocytes from a patient affected with Hodgkin's disease show the virus-like particles to be spherical with irregular contour, and to be on or somewhat in the erythrocyte. These virus-like particles have a diameter of 110 to 120 mu. The bodies could not be demonstrated in normal human blood subjected to the same procedure of preparation and electron microscopic examination. These bodies resemble the virus of measles and the virus bodies of infectious mononucleosis described by Reagan *et al.* (3) (4)

FIG. 3. Electron micrograph of an erythrocyte from a healthy normal adult. Shadowed with chromium at arc tangent 4/14. x 40,000.

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PRIMARY DISEASE PROCESSES AS OBSERVIED AT NECROPSY IN 353 PEDIATRIC CASES EXAMINED BETWEEN 1948 AND 1952 AT THE MEDICAL BRANCH OF THE UNIVERSITY OF TEXAS*

M. THOMSEN AND R. H. RIGDON

Data obtained from postmortem examinations will indicate some of the problems referable to disease and some of the medical problems that are peculiar to the region where such studies are made. Such information may be significant when the number of postmortem examinations are 50 per cent or more of the total deaths occurring in an institution. In this study we have reviewed 1500 routine necropsies performed between July 2, 1948 and September 14, 1952. Of these, there were 353 (23.5%) in children fifteen years of age or younger. This number represents approximately 75 per cent of the deaths occurring on the Pediatric Service at the Medical Branch of The University of Texas during this period. The age, sex and race of these cases are shown in Table 1. It is of interest to note that 211 of these were whites and

TABLE 1

Age, sex and race of 353 necropsied case of individuals less than 16 years of age

Age	WF	WM	CF	CM	Total
Stillborn	4	2	7	9	22
Newborn	31	33	17	26	107
5 wk.—6 mo.	23	21	6	17	67
7—12 mo.	8	5	5	4	22
1—2 yr.	6	14	4	9	33
3—5 yr.	10	15	4	7	36
6—10 yr.	8	11	11	6	36
11—15 yr.	5	15	4	6	30
Total	95	116	58	84	353

142 Negroes. There were 200 males and 153 females. The majority of these necropsies were performed upon newborns and infants four weeks of age or younger. The number of necropsies was approximately the same for the age groups, 1–2, 3–4, 6–10 and 11–15 years of age.

* From the Department of Pathology, The University of Texas Medical Branch, Galveston, Texas. Submitted for publication November 11, 1953.

TABLE 2

Primary pathologic diagnosis in 353 cases

68 Central Nervous System
16 Malformations
15 Meningitis-Pyogenic
14 Meningitis-Tuberculous
1 Brain abscess with otitis
1 Thrombus in meningeal vessels
1 Poliomyelitis
8 Glioma
1 Pinealoma
1 Cyst in third ventricle
1 Hemangioma, roof of 4th ventricle
1 Cerebral palsy
1 Schilder's disease
7 Subdural & Subarachnoid hemorrhages

20 Infectious Diseases
5 Diarrhea—Etiology?
3 Tetanus
2 Generalized tuberculosis (without meningitis)
2 Umbilical sepsis
1 Chronic colitis—Etiology?
2 Staphylococcus albus sepsis
1 Meningococcus—Waterhouse Friedrichs syndrome
1 Osteomyelitis of skull
1 Congenital sepsis
1 Histoplasmosis
1 Fungus—type?

14 Congenital Lesions—Miscellaneous
4 Multiple congenital lesions
2 Hare lip and cleft palate
2 Mongoloid idiot
2 Omphalocele
1 Nasal obstruction, congenital
1 Micrognathia
1 Absence of left diaphragm
1 Dyschondroplasia calcificans

28 Hemopoietic System
16 Leukemia
5 Sickle cell anemia
2 Erythroblastosis fetalis
2 Anemia-type undetermined
1 Aplastic anemia
1 Hemorrhagic diathesis
1 Hodgkin's Disease

15 Genito-Urinary & Adrenals
2 Wilm's tumor
6 Neuroblastoma
1 Congenital cystic kidneys
4 Nephritis
1 Adrenal Hypoplasia
1 Hemorrhage into adrenals

39 Cardio-Vascular System
29 Congenital heart disease
6 Rheumatic heart disease
1 Diffuse myocarditis, etiology?
1 Maffucci's syndrome
1 Fibro-elastosis
1 Coronary occlusion

19 Gastro-Intestinal System
6 Imperforate anus
5 Tracheo-esophageal fistula
1 Atresia of jejunum
1 Strangulated hernia
1 Fibrocystic disease
1 Ruptured appendix
1 Pyloric stenosis
3 Stricture of esophagus (lye)

4 Liver and Biliary System
2 Neoplasm of liver
2 Banti's syndrome

34 Respiratory System
26 Pneumonia
4 Congenital atelectasis
3 Aspiration of amniotic fluid
1 Congenital cystic lungs

36 Miscellaneous
19 Burns and trauma
4 Glycogen & lipoid storage diseases
3 Undetermined
2 Dwarf
2 Osteosarcoma
3 Sarcoma, sites undetermined
1 Diabetes
1 Exsanguination from umbilical cord
1 Still's Disease

59 Prematures
32 Prematurity
18 Subarachnoid hemorrhage
4 Pneumonia
3 Hyaline membrane disease
1 Multiple lung abscesses
1 Melena Vera

17 Stillbirths
10 Undetermined
2 Erythroblastosis fetalis
1 Medulloblastoma
1 Glycogenic tumor of heart
1 Prolapsed cord
1 Traumatic
1 Ancephalic Monster

The basic pathologic process in these 353 cases is shown in Table 2. There were 74 congenital lesions, 20.9 percent; 51 infections (other than penumonia), 14.4 per cent; 26 neoplasms, 7.3 per cent; 19 traumatic, 5.5 per cent and 183 miscellaneous cases, 51.8 per cent. The central nervous system was primarily involved in 68 cases, as shown in Table 2. This system was more frequently involved than any other. There were 39 cases involving the cardiovascular and 34 the respiratory systems.

There were 74 cases (20.9%) with malformations. Twenty-nine of these involved primarily the heart; 16, the central nervous system; 13, the gastro-intestinal tract; one the lungs and one the kidneys. In 14 cases the malformation included more than a single system.

There were 26 cases (7.3%) with neoplasms. The central nervous system had a total of 11 tumors; adrenal, 6; kidneys, 2; liver, 2; bones, 2 and 3 were located in unspecified sites. The most frequent neoplasm was a glioma, of which there were 8. Neuroblastomas were second with a total of six cases. There were three sarcomas apparently arising from soft tissues.

The group of infectious diseases, other than pneumonia, constitutes one of the most interesting in this study. There was a total of 51 cases (14.4%). Sixteen of these had tuberculosis; 15 had a pyogenic infection of the central nervous system; 5 infantile diarrhea; 3 tetanus and 2 umbilical sepsis. The other types of infections are shown in Table 2. In the fifteen cases of pyogenic meningitis the etiologic agent was as follows: 4 pneumonococcus, 4 influenza bacillus, 1 streptococcus, 1 B. coli and 5 were undetermined.

In reviewing the clinical data on these cases with infections other than tuberculosis, it was found that the average stay in the hospital was 2.5 days; the longest was 16 days and the shortest 10 minutes. Twenty-five of these cases remained in the hospital 48 hours or less. The records show that these children were sick for an average of 6.8 days before hospitalization. The longest time was 30 days and the shortest was 8 hours. There were 18 cases in the Negro race and 13 in the White race.

In the 16 cases with tuberculosis, the age range was 6 months to 14 years. The infection was widespread in 14 cases. The meninges were also involved in 14 cases. Tuberculomas were present in the brain of one case without meningitis. The brain was not

examined in one case with extensive tuberculosis. Six of these cases of tuberculosis gave a history of a positive contact. Eleven cases occurred in the White (including Latin-Americans) and 5 in the Negro races. The length of illness preceding hospitalization varied from 4 hours to 9 months with an average of 43.7 days.

Discussion. In compiling these statistics on the cause of death the 17 cases of stillborns and the 59 prematures were not included. The anatomical cause of death in a high percentage of the premature births was not determined. In our opinion, many such cases do not show anatomical changes that will adequately account for their demise. The anatomical diagnosis of "hyaline membranes" will vary widely with different pathologists. No doubt such lesions could be demonstrated more frequently than these data would indicate; however, the fact would remain as to whether the presence of this membrane could be considered the primary cause of death, rather than some physiologico-chemical mechanism accompanying the prematurity influencing the development of this membrane.

The absence of congenital syphilis (except for one) in this group of cases is very interesting, thus indicating what has been accomplished in prenatal care of syphilitic mothers. There are only two cases of neoplasms that may be considered as an "embroyonic type." These were in the kidneys. There may be a variation in the opinion referable to the neoplasm observed in a stillborn; however, several pathologists have considered this most likely a medullo-blastoma. Kidder (1950) reviewed the literature on glycogenic tumors occurring in the heart and reported one of these cases. Willeford and Stembridge (1950) have reported the case of hepatic sarcoma that occurred in a 6 year old white child included in this series. They pointed out the difficulty in the diagnosis of such neoplasms arising in the liver.

The many deaths from tuberculosis in these children are indicative of the need for isolation of known cases of this infection. The therapeutic agents now used in the treatment of tuberculosis would appear to be inefficient in some cases. Regardless of the availability of many antibiotics for the treatment of infectious diseases, the delay before hospitalization is still an important factor in the successful treatment.

The high percentage of deaths in this group of necropsies on stillborns and premature infants would indicate the need for further clinical study in its prevention.

Summary. This review of the pediatric cases studies at necropsy at the Medical Branch Hospital between 1948 and 1952 shows that in this institution tuberculous meningitis still is an important disease. The length of illness and the duration of hospitalization of such individuals present important medical and economic problems.

Deaths from infections, other than tuberculosis, in this study would suggest that the patients had a virulent infection and were very ill when admitted to the hospital. The average stay was only 2.5 days, with the longest 16 days and the shortest 10 minutes. Furthermore, these cases were sick for an average of 6.8 days when first hospitalized in our institution.

Malformations, as one would expect, were frequent (20.9%). The cardiovascular and central nervous system and the gastrointestinal tract were the three sites most frequently involved.

Neoplasms accounted for the deaths of 7.3% of the children. A majority of these tumors occurred in the brain and in the adrenals.

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STUDIES ON SKIN REACTIONS AND PRECIPITIN ANTIBODIES PRODUCED AGAINST CERTAIN ATOPIC AGENTS BY CITELLUS TRIDECEMLINEATUS TRIDECEMLINEATUS

ROBERT L. TIPS*

INTRODUCTION

The effect of atopic agents upon the susceptible human subject is well known. A given atopic agent such as grass pollen or ragweek pollen has been shown to induce at least three types of antibodies; the reaginic antibodies, inhibiting antibodies (Cooke *et al.*, 1935; Loveless, 1940; Scully and Rackemann, 1940–41), and precipitin antibodies (Munoz and Becker, 1949; Strauss and Spain, 1940–41). The physiological effects of the reactions of the agents and specific antibodies are well known clinically.

The effects of atopic agents in animals is not well known. The precipitins induced by these agents has been the subject of many diverse investigations. Chase (1943), Reddin (1948–50), and Wittich (1940–41) have presented evidence of reagin production induced by atopic agents in some mammals.

It might be expected that the introduction of a given atopen into the tisues of individuals of the same species should elicit the production of similar types of antibodies in all members of that species. It is the purpose of this investigation to study skin reactions and precipitin reactions of the thirteen lined ground squirrel, *Citellus tridecemlineatus tridecemlineatus* induced by grass and ragweed pollen atopens.

Methods. Citellus tridecemlineatus tridecemlineatus was chosen for this study because of its local availability in wild populations. More than 100 ground squirrels were taken in three separate collections.

Collections were made from a ten acre grass surfaced area uninterrupted by any physical barriers. The captured animals were placed in wire rat

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cages in an animal room. All were fed Rockland Farms Rat Diet and water *ad libitum*.

TABLE 1

The number of animals taken in the collection

Collection	Dates of Collection	Males	Females
I	April 20–22	24	4
II	May 1 & 2	12	30
III	Sept. 9	17	15
Totals		53	49

Most of the animals were considered to be sexually mature at the time of capture. All of the animals of the first and second collection were at least one year old. Nothing is known of the age of the third collection animals except that they were old enough to be sexually mature. Only twenty-eight females and thirty-nine males survived in captivity during the course of this work.

Skin Tests. Shortly after collections were completed, the entire back of each animal was shaved (further shaving during the course of experimentation was not necessary because new fur was not produced). Subcutaneous injections of 3% ragweed pollen extract and of a 3% grass pollen extract (obtained from Abbott Laboratories) were employed to test the skin reactions of each animal to these atopens. The injection of 0.01 ml. of an extract was sufficient to produce a wheal in the skin. Typical positive skin tests (as those known in man) could not be obtained after five attempts to test the animals. It was noted, however, that certain animals showed a small dark red spot beneath the wheal. This at first was considered to be a small hemorrhage caused by the injection. It did not spread, nor was the skin reddened. Water and saline control injections did not produce this effect. This "hemorrhage" reaction was thought to be a specific reaction to the atopen. To test further, passive transfer was attempted.

Ten non-reactive males were used for all passive transfer work, and were given skin tests at intervals to determine if they had become reactive to the pollen extracts.

About 2.0 cc. of blood was drawn from the heart of each of ten other animals selected at random from the collection. The serum was collected in the usual manner, diluted and mixed with the atopens. Each serum was diluted into a set of test tubes with physiological saline.

TABLE 2

Serum-extract mixtures for use in passive transfer tests

	Test Tube Numbers								
	1	2	3	4	5	6	7	8	9
Serum dilution	1/1	1/1	1/10	1/20	1/40	1/1	1/10	1/20	1/40
Ragweed extract	0	0.1	0.1	0.1	0.1	0	0	0	0
Grass extract	0	0	0	0	0	0.1	0.1	0.1	0.1

One hundredth milliliter of the contents of each tube was injected into marked sites on the backs of the selected males. It was found that certain sera produce the same "hemorrhage" reaction as in the skin tests. This reaction represents a positive passive transfer test. It was also noted that only the skin "positive" animals (those giving the "hemorrhage" reaction when injected with the allergen) were able to transfer their reaction. Serum dilutions beyond 1/10 gave no reaction. However, all undiluted serum that produced a "positive" reaction gave one also in a 1/10 dilution. The explanation for this is not obvious. Serum alone produced no reaction.

The ten passive transfer animals were tested to determine if each could accept the "passive transfer" from a known positive serum. A sample of ragweed extract and of grass extract were each diluted with an equal amount of 1/10 diluted positive serum. A mixture of equal parts saline and diluted positive serum served as control. One-hundredth milliliter of each mixture was injected into one of the three marked sites on the shaved back of the animal to form a wheal. It was found that all ten animals could receive the "passive transfer." Using these acceptors, passive transfer tests, as described for the ten selected at random, were done on all animals of the experimental group. All tests were repeated several times.

It was found that skin tests parallel exactly the passive transfer tests. Each animal showing a positive skin test had a passive transfer titer of 1/10; negative animals gave no passive transfer reaction. Thus these two tests may be considered as an expresssion of a single positive skin reaction.

In order to insure that the animals had received sufficient atopen to elicit a positive skin reaction, each animal was given a series of intradermal injections of the same grass and ragweed pollen extracts used for skin tests. Injections of 0.5 cc. of each extract were administered at one week-intervals for five weeks. Skin tests and passive transfer tests were performed several times during and after the injection series. All animals giving positive skin tests also gave passive transfer titers of 1/10. All animals with negative skin tests did not possess a detectable passive transfer titer.

Results

The responses to the extracts used are presented in Table 3. Six completely negative animals became positive reactors; five animals exhibited positive reactions to both extracts. Three animals remained positive to grass extracts and six to ragweed extracts. These changes occurred after the first week of treatment. In no case did a positive reaction become negative before or after treatment. Attempts to induce changes using much larger doses of the extracts usually resulted in the death of the animal, possibly due to anaphylactic shock.

Serum was obtained from three animals positive only for grass pollen and from six animals positive only for ragweed pollen. These sera did not give a detectable positive passive transfer re-

action when mixed with the respective heterologous pollen extracts. The reagin-like antibodies are thus apparently specific for the pollen extracts.

TABLE 3

The distribution of animals giving positive and negative skin reactions before and after the injection series treatment

	Positive for both extracts	Positive only for grass extracts	Positive only for ragweed extracts	Negative for both extracts
Before treatment	12	4	4	36
After treatment	17	3	6	30

Precipitating Antibodies Produced Against the Pollen Extracts. Shortly after the collections were completed, serum was obtained from sixty-six animals and was stored in individual test tubes at 10° C. until examined three or four days later.

A flocculation test modified from Cannon and Marshall (1940) was employed to ascertain precipitin titers for each serum. Colloidal collodion particles were prepared according to prescribed directions and were divided into two lots. Ragweed pollen extract was added to one suspension and mixed grass pollen extract to the second suspension. When the flocculation tests were performed, non-specific flocculation occurred in the colloidal antigen controls. It was found that this flocculation did not occur when 0.42% saline was used as the diluent rather than 0.89% saline.

The serum agar technique described by Becker and Munoz (1950) was also employed to determine precipitin titers. Filtered Difco agar was adjusted to approximately pH 7.0; serum and the diluted pollen extracts were prepared. Precipitin titers were read after one week incubation at about 35° C. instead of reading after two weeks incubation at 25° C. because no difference had been found in several readings when the two incubation temperatures and times were used.

No difference could be detected in determining the presence and absence of specific precipitation by the two methods. In some cases the two methods did not give identical titers for the same positive sera but both gave titers within two dilutions (a double dilution scheme was used in both methods). Although the pollen extracts are doubly diluted in the test tube series of the agar

method and the serum is doubly diluted in the colloidal method, neither method gave consistently higher or lower titers for reactive sera.

The distribution of animals according to titer of precipitin reaction is given in Table 4 before and after injection treatment described under skin tests. Forty of the fifty-six animals of the experimental group failed to give any detectable precipitin reaction and are not included in the table. For the sake of simplicity the term "low titer" is used to indicate dilutions between 1/10 and 1/40, "intermediate titer" dilutions between 1/40 and 1/160, and "high titer" dilutions above 1/160.

TABLE 4

The distribution of animals according to grass and ragweed pollen precipitin titer before and after injection series

	High titer		Intermediate		Low titer	
	Before	After	Before	After	Before	After
Grass and ragweed	1	4	2	0	2	0
grass pollen only	0	6	3	0	3	0
ragweed pollen only	0	6	3	0	3	0

Unlike the effect on skin reactions, the injection series failed to induce precipitin antibody production in any individual that did not previously give a detectable precipitin titer. In fact, the only noticeable effect of the treatment was to raise the titer greatly in those animals that gave a titer previously. In the three animals producing precipitins to grass pollen extract the injection treatment with both extracts raised the titer against grass but did not allow the production of precipitins against ragweed pollen extract. The same is true for those producing precipitin to only ragweed pollen. Furthermore, the injection treatment allowed the titer against both atopens to increase no matter what differences between the two titers existed previously.

The precipitin tests after treatment were carried out six to eight weeks after treatment had begun. Further attempts to produce precipitin titers in non-producing individuals failed. Only one member of the passive transfer test males gave a precipitin reaction. It possessed a low titer for grass extract and a high titer for ragweed pollen extract.

Correlation of Skin Test and Precipitin Titer Data. The reagin-producing haptenes and the precipitin antibody-producing hap-

tenes of the pollen proteins should be present in all cases where pollen extracts are used. It would be expected that any individual producing one type of antibody would also produce the second type. This was not found to be the case. Data are presented in Table 5 showing the distributions of the positive and negative skin tests among individuals giving precipitin titers (reactive) and those not giving a detectable precipitin titer (non-reactive). Approximately one-third of the skin test positive animals are reactive for the precipitin test and approximately one-fourth of the skin test negative are reactive.

TABLE 5

Distribution of skin reactions among precipitin reactions before and after injection treatment

1. Before treatment Skin tests	Precipitin tests				
	Reactive to ragweed and grass	Reactive to grass only	Reactive to ragweed only	Non-reactive ragweed and grass	Total No.
Positive to grass and ragweed	0	0	4	8	12
Positive to grass only	0	0	1	3	4
Positive to ragweed only	0	0	1	3	4
Negative to ragweed and grass	4	6	0	26	36
Total	4	6	6	40	56
2. After treatment					
Positive to grass and ragweed	1	0	4	12	17
Positive to grass only	0	0	1	2	3
Positive to ragweed only	0	1	1	4	6
Negative to ragweed and grass	3	5	0	22	30
Total	4	6	6	40	56

Thus it appears that under the experimental conditions of this work, there is no relation between an individual's capacity to produce the skin sensitizing antibodies and its capacity to produce the precipitin.

Discussion. The skin tests and passive transfer tests indicate that the strange "hemorrhage" reaction on the injected skin wheal is specific for one or the other of the pollen extracts. The fact that this reaction may be passively transferred gives strong evidence of its antigen-antibody nature. Thus it is possible to refer to such a reaction as an atopen-reagin reaction.

Whether the stimulation of the reagin-like antibodies was obtained from the atopens in nature or from atopens used in the preliminary skin tests could not be determined. However, the injection treatment allowed the reaction to be produced by a few previously negative animals.

It may be noted that only certain individuals could be induced to produce the skin reactions. The reasons for this are unknown, but the data would not be incompatible with an hypothesis of an hereditary factor. It may be further noted that certain animals can be induced to give only one or the other skin reaction but not both. Other animals can be induced to give both reactions.

The evidence that certain individuals show only one skin reaction and not the other gives strong support to the suggestion that the two reactions are immunologically distinct and specific. This suggestion is also strongly supported by the fact that positive skin reactions cannot be passively transferred by heterologous atopens.

The data on precipitin tests indicate that ability to produce detectable precipitating antibodies is restricted only to certain animals. The reason for this cannot be ascertained from the data. The injection treatment appeared merely to raise the titer of the reactive animals but did not induce non-reactive animals to produce detectable precipitin antibodies.

Some reactive individuals were capable of producing precipitin only to one or the other pollen extract. Although the injection treatment did not induce any change in this capacity, these observations indicate that the precipitin reactions with ragweed and grass pollen extracts are immunologically distinct and specific.

The distribution of skin reactions among the precipitin tests for both pollen extracts does not indicate that there is any regular relation between the observed capacities for these two types of apparently immunologically distinct antibodies produced by

the animals. The injection treatment does not change the distributions.

Recently Loveless and Conn (1953) have shown that reagins to different allergens are located in different human serum fractions. They have also demonstrated that reaginic and blocking antibodies are essentially different chemically, physically, and immunologically.

The pollen extracts used in this work are the same extracts that have been used for desensitization purposes in humans. This type of material was used by Loveless (1940) in the demonstration of the inhibiting or "immune" antibodies in humans. No data are presented that give any indication as to the implication of this fact in the strange "hemorrhage" skin reactions obtained. If these skin reactions are true antigen-antibody reactions it would seem that the antibodies produced are reagin-like antibodies.

Summary. An unusual "hemorrhage" skin reaction was obtained by subcutaneous injections of ragweed and grass pollen extracts into *Citellus tridecemlineatus tridecemlineatus*. Passive transfer tests indicate this reaction to be an atopen-reagin reaction. Evidence was presented that these reactions are immunologically distinct and specific for each pollen extract. The positive skin reactions were restricted to a few individuals in the animal colony. Certain of these positive animals produced reactions only to one or the other pollen extracts but not to both. Other positive animals reacted to both extracts. No essential change in the capacity to produce these skin reactions was noted after large dosse of both extracts had been given to each individual, except a few previously negative individuals gave typically positive reactions.

Precipitin antibodies can be produced by certain individuals of the animal colony. The injection of large doses of the extracts served only to increase titers but did not change the inability of negative animals to produce precipitin. No regular immunological relations were found between skin tests and precipitin tests.

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SECONDARY GROWTH IN *TETRAHYMENA GELEII (Y)*

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Most studies of the growth of microorganisms have been concerned with the earlier phases of the histories of cultures—the lag, logarithmic, and stationary phases (Van Niel, 1949)—and relatively little attention has been paid to the declining phases of a culture. Secondary growth has been reported in the cases of some species of bacteria; this is due to an adaptation (Lodge and Hinshelwood, 1944) or mutation (Fildes and Whitaker, 1948) which overcomes a nutritional limitation of the medium. Richards (1932) reported a similar phenomenon in yeast cultures; this was explained as being due to the killing of the cells as fast as or faster than new cells were formed by the alcohol produced by fermentation during initial growth. When the metabolism of the surviving cells had reduced the alcohol concentration below the toxic level, growth was resumed. In all these cases, growth curves similar to that diagramed in Figure 1 were obtained.

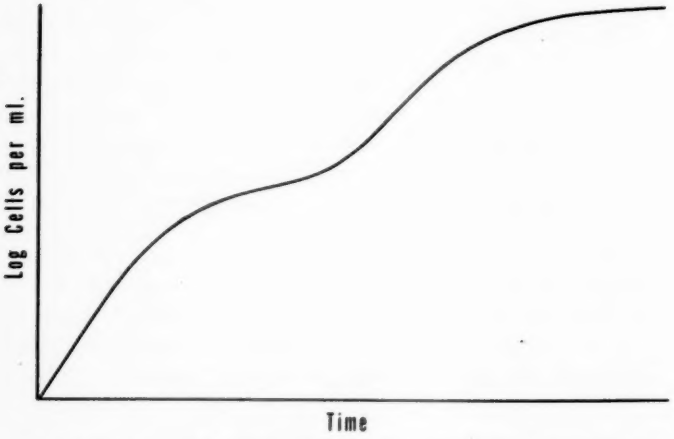

FIG. 1. Growth curve for bacteria or yeast showing secondary growth phase.

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Recently, in some studies in which cultures of *T. geleii (Y)* were followed past the time of maximum population, the authors have encountered a somewhat similar phenomenon; it consists of a rapid decline in numbers of cells in the culture, followed by a partial recovery. Some striking morphological changes are associated with these changes in population.

Experimental. Cultures of *T. geleii (Y)* were grown in the medium previously used by Wingo and Cameron (1952); this is essentially Phelps' (1935) medium, modified by the inclusion of extra phosphate buffer (0.05M, pH 7.3), containing five times the usual quantity of calcium chloride and with 1% Difco Proteose-Peptone instead of yeast extract. The medium was neither filtered nor centrifuged before autoclaving, and on autoclaving a considerable quantity of finely-divided amorphous precipitate separated. The medium was dispensed in quantities of 100 ml. in culture vessels similar to those described by Browning *et al.* (1952), and the vessels, after inoculation, were incubated, under aeration, in a water bath at 37° C. (The water bath was protected from bright light.)

Counts were made at four-hour intervals, using a Segewick-Rafter counting cell in connection with a microscope equipped with a calibrated Whipple eyepiece micrometer. One hundred fields were counted for each sample; the fixing fluid was a 5% solution of formalin, the diluent being so made up that the final fixative had the same electrolyte composition as the medium (except for the contribution of the Proteose-Peptone). When dilution became necessary, the fixative was used as diluent, and the volume ratios were so chosen that about 1,000 cells were counted in each 100 fields.

Results and Discussion

The results, as regards the numbers of cells per milliliter, are shown in Figure 2.

The initial growth phase was very rapid, the peak population being reached approximately 50 hours after inoculation. During this time, the cells were "normal" in appearance, and were approximate ellipsoids of revolution, about 45–50 microns long and 20 microns in diameter. With the onset of the first phase of senesence, the morphology of the cells underwent a drastic change; they became smaller and more nearly round, until at about 70 hours by far the greater part of the cells counted were almost spherical, and not over 20 microns in any dimension. A considerable fraction of the cells were, during this phase, beginning to lyse when seen in the counting chamber, and no division figures were seen.

When the culture density had fallen to about 10% of its maximum value, "normal" cells began to increase in relative number, and cells in division were seen. This process continued for approximately 15 hours during which the density of the culture almost doubled, and the morphologically abnormal cells had almost disappeared. A second, slower phase of death then set in.

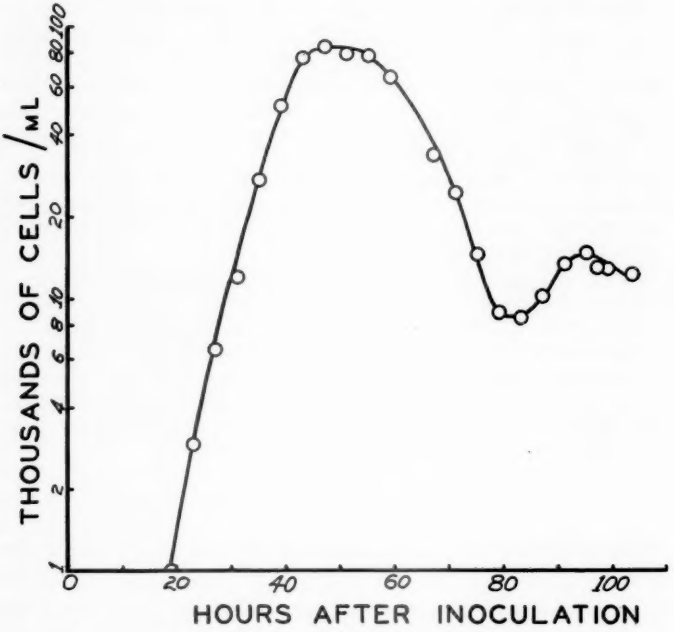

FIG. 2. Initial growth, phase of rapid death, and secondary growth of *T. geleii (Y)*.

Similar results have been obtained in similar cultures fortified with thiamine, the main differences being that much higher levels of population were obtained in this case, and that after the peak population was reached some extremely bizarre involution forms appeared and continued throughout the rest of the experiment.

In similar experiments in which the medium had been autoclaved, clarified by centrifuging, dispensed in the culture vessels,

and autoclaved again, what the authors believe to be the same phenomenon was encountered. The initial death phase was not so striking, but the recovery from it was unequivocally demonstrable.

The causes and mechanism of the initial phase of rapid death and of the phase of secondary growth reported here remain unknown. At least three possibilities must, however, be considered.

(1) The first death phase may be terminated by the appearance of a mutant strain of protozoa capable of at least limited synthesis of the nutrient whose exhaustion has led to the decline of numbers of cells.

(2) A non-mutative adaptation to synthesize the critical nutrient may occur.

(3) Sufficient critical nutrient to arrest the decline of the population and to bring about a marked secondary growth may be furnished the surviving cells by the autolysis of the cells which die during the initial death phase.

Summary. The occurrence of a secondary phase of growth, following the rapid initial death phase in cultures of *T. geleii(Y)* is described, together with the concomitant morphological changes in the protozoan cells. Three possible mechanisms for this phenomenon are considered.

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CALLING ATTENTION TO:

Items of Possible Interest to Our Friends

Happy Holidays, 1953

Preclinical: N Arley & S Iversen study quantum hit theory of carcinogenesis (*Acta Path Microbiol Scand* 30/21/52; 31/164/53; 33/133/53). . . . F. Challenger surveys biological importance of organic sulfur compounds (*Endeavor* 12/173/53). . . . MV Dianzani notes lipotrpotein mitochondrial membrane (*Experient* 9/343/Sept 15/53). . . . JC Fascicle describes angiediactin, a vasodilator from renal extracts (*Acta Physiol Lat Amer* 3/1/53). . . . HH Fox discusses new chemical types of tuberculestatic drugs (*Science* 118/497/Oct 30/53). . . . HP Kaufmann reviews Arzneimittel-Synthese (Springer, Berlin, 53, 834pp, 87 DM). . . . PF Schelander & Co. report studies on physiology of frozen Artic plants & animals (Suppl 1, vol 42, *J Sell Comp Physiol*, 56pp, Sept 53). . . . L Steckinger discusses function of nucleolus (*Protoplasma* 42/365/53). . . . V du Vigneaud & Co announce synthesis of oxytocin & complicated structure of vasopressor & antidiuretic hormone of pest-pituitary (*J Am Chem Sec* 75/4878, 4880/53). . . . F Windisch & D Streiand discuss cell phosphate & lack of specificity of histochemical localization of ribonucleotides (*Protoplasma* 42/345/53).

Clinical: DD Adams suggests allergic persons have abnormally responsive antibody-forming mechanisms (*Lancet* 2/911/Oct 31/53). . . . BM Evans & Co recommend Na Sulfonic ion exchange resin orally in oliguria with hyperkalemia (*Lancet* 2/791/Oct 17/53). . . . IRS Gorden discusses current cholecystography (*Quart J Med* 22/261/53). . . . KF Meyer reviews vectors & reservoirs of virus diseases (*Amer J Trop Med Hyg* 2/757/53). . . . JH Meyer & I Johnson say hypertension is helped by IM injections of water soluble standardized mixed ester alkaloids of veratrum ("veroloid") (*Am J Med Sci* 226/477/53). . . . W Requarth helps in Diagnosis of *Acute Abdominal Pain* (Year Book Publ, Chicago, 53, 243pp, $5). . . . Our A Ruskin well discusses *Physiological Cardiology* (CC Thomas, Springfield, Ill, 53, 370pp. $6). . . . A Studer gives full study on action of cortisone (*Ztschr ges Exp Med* 121/287–418/53). . . . JM Vandepitte & LA Louis differentiate sickle cell anemia from trait by bone marrow smears (*Lancet* 2/806/Oct 17/53). . . . W Wilbrandt describes mechanisms of hemolysis (*Schw Med Wzhnschr* 83/1023/Oct 24/53). . . . J Wilder claims some benefit in multiple sclerosis with iv injections of heart muscle extract digested with pancreas & phenol (*Conf Neurol* 13/222/53).

Psychology & Psychiatry: J Adelson studies minority group authoritarianism (*J Abn Soc Psych* 48/477/53). . . . JC Carothers looks at *The African Mind in Health & Disease: A Study in Ethnopsychiatry* (WHO Monograph Ser 17, Geneva, 53, 177pp). . . . Note Editorial on medical & non-medical psycho-therapy, a plea for licensing non-medical clinical psychologists (*Am J Psychother* 7/1/53). . . . GWTH Fleming discusses "The Insane Root" (mandrake) for centennial issue *Journ Ment Sci* (99/638/53).

. . . EM Jones begins 3 vol *Life & Work of Sigmund Freud* (Vol 1, Basic Books, NY, 53, 353pp, $6.75). . . . SH Kraines & ES Thetford add to auto-perceptive therapy in *Live & Help Live* (Macmillan, NY, 53, 408pp, $3.95). . . . Importantly LS Kubie notes unsolved psychological problems of scientific careers (*Amer Sci* 41/596/53). . . . HC Lehman appraises *Age & Achievement* (Princeton Univ., Princeton, NJ, 53, 378pp, $7.5). . . . VE Lehmann & FA Risquey describe qualitative & quantitative use of finger painting in clinical appraisal of psychotic conditions (*J Ment Sci* 99/763/53). . . . WL Neustatter relates *Psychological Disorder & Crime* (British Book Centre, NY, 53, 248pp, $4.75. . . . CD Oberndorf recounts the *History of Psychoanalysis in America* (Grune & Stratton NY, 53, 323pp, $5). . . . W Overholser considers *The Psychiatrist & the Law* (Harcourt, Brace, NY, 53, 134pp, $3.5). . . . F Redlich & J Bingham give *The Inside Story* of personality development by cartoon & quip (Knopf, NY, 53, 96pp, $3). . . . G Saenger discusses *The Social Psychology of Prejudice* (Harper's, NY, 53, 187pp, $4). . . . JP Sartregoes for *Existential Psychoanalysis* (Philosophical Library, NY, 53, 210pp, $4.75). . . . R Straus & SD Bacon report on *Drinking in College* (Yale Univ Press, New Haven, Conn., 53, 270pp, $4).

GENERAL: Dean Burk well eulogizes Otto Warburg, artisan of cell chemistry, on his 70th birthday (*Biochem Biophys Acta* 12/9/53, pp 9–359 review current enzyme chemistry). . . . SJ Hadfield offers field survey of British general medical practice (*Brit Med J* 2/683/Sept 26/53). . . . J Mayer discourses on mice disrespectful of obesity (Harper's, Oct/53, p81). . . . R Moore tells of *Man, Time & Fossils* (Knopf, NY, 53, 411pp, $5.75). . . . RL Roy describes *Apostles of Discord* (Beacon Press, Boston, 53, 440pp, $3.75). . . . K Shapire gets *Beyond Criticism* in poetical analysis (Univ Nebraska Press, Lincoln, 53, 167pp, $3).

NEW YEAR, 1954

GENERAL: RH Bainton sympathizes with *Hunted Heretic: The Life & Death of Michael Servetus (1511–1553)* (Beacon Press, Boston, 53. 270pp, $3.75). . . . SJ Beck discourses on *The Science of Personality* (*Psychol Rev* 60/353/53/). . . . EM Bell describes *Storming the Citadel: The Rise of the Woman Doctor* (Constable, London, 53, 200pp, 18s). . . . RB Braithwaite is tough in *Scientific Explanation: A Study of the Function of Theory, Probability & Law in Science* (Cambridge Univ Press, London, 53, 267pp. 21s). . . . KJ Franklin well biographizes *Joseph Barcroft 1872–1947* (Blackwell, Oxford, 53, 400pp. 37s 6d). . . . F Fremont-Smith well edits *Health & Human Relations* (Blakiston, Garden City, NY, 53, 228pp, $4). . . . M Kline analyses *Mathematics in Western Culture* (Oxford Univ Press, NY, 53, 360pp. $7.5). . . . LS Kubie concludes study of unsolved problems of scientific careers by referring to socia-economic matters (*Amer Sci* 42/104/54). . . . A Malraux psychologizes art in *The Voices of Silence* (Trans. S Gilbert, Doubleday, NY, 53, 661pp, 465 illus, $25). . . . O Ore interests in *Cardano, the Gambling Scholar* (Princeton Univ Press, Princeton, NJ, 53, 226pp, $4). . . . DW Richards discusses homeostasis versus hyperexis pleasantly (*Sci Month* 77/289/53). . . . A Roe looks to *The Making of a Scientist* (Dodd, Mead, NY, 53,

253pp, $3.75). . . . DW Singer discusses science & confidence in the rational mind (*Centaurus* 3/166/53). . . . SM Spencer describes some more *Wonders of Modern Medicine* (McGraw-Hill, NY, 53, 299pp, $4).

CLINICAL: CH Ammons & Co find that blinded but otherwise healthy persons can learn to perceive obstacles (*Am J Psychol* 64/519/53). . . . J Bock & Co again show tincture of iodine is superior to mercury antiseptics for sterilizing skin (*Acta Pharmakol Toxicol* 9/201/53). . . . JJ Bonica offers *The Management of Pain* (Lea & Febiger, Phila, 53, 1533pp, $20). . . . CM Landmesser & Co discuss respiratory stimulating action of N-allyl normorphine in morphine depression (*Anesth* 14/535/53). . . . JM Lord, BW Powell & H Roberts say deaths from post-natal asphyxia are preventable, & recommend planned & practiced resuscitation technic, prompt aspiration, endotracheal insufflation for mature infants & gastric oxygen for small prematures (*Lancet* 2/1001/Nov 14/53). . . . FC MacGregor & Co make a psychosocial study of *Facial Deformities & Plastic Surgery* (CC Thomas, Springfield, Ill, 53, 244pp, $5). . . . W Marshall encourages curiosity & investigation in general practice in *Essentials of Medical Research* (Vantage Press, NY, 53, 176pp, $3). . . . DR Miller discusses prediction of behavior by Rorschack testing (*J Abn Soc Psych* 48/367/53). . . . EW Page describes *The Hypertensive Disorders of Pregnancy* (CC Thomas, Springfield, Ill, 53, 134pp, $3). . . . R Pullen edits *Pulmonary Diseases* (Lea & Febiger, Phila, 54, 700pp, $14). . . . GF Reddish edits *Antiseptics, Disinfectants, Fungicides, & Chemical & Physical Sterilization* (Lea & Febiger, Phila, 54, 510pp, $10). . . . JD Russ discusses *Resuscitation of the New Born* (CC Thomas, Springfield, Ill, 53, 63pp, $2). . . . CR Spealman edits *Aviation Toxicology* (Blakiston, Garden City, 53, 120pp, $4). . . . E Tolstoi describes *The Practical Management of Diabetes* (CC Thomas, Springfield, Ill, 53, 103pp, $3). . . . CA Whitaker & TP Malone examine *The Roots of Psychotherapy* (Blakiston, Garden City, NY, 53, 236pp, $4.5). . . . E Ziskind offers *Psychophysiologic Medicine for General Practitioners* (Lea & Febiger, Phila, 54, 360pp, $7).

PRECLINICAL: F Barron proposes an ego-strength scale to predict response to psychotherapy (*J Consult Psych* 17/327/53). . . . GH Bourne & HA Malaty find cortisone increases succinic dehydrogenase in mammalian organs (*J Physiol* 122/178/53). . . . IA Boyd & TDM Roberts note proprioceptive sense organs in knee joints controlling fine movements (*J Physiol* 122/38/53). . . . W Gooddy & M Reinhold offer gestalt theory of sensations, notions of time & space, & abstract thinking with nervous system orientation of space (*Brain* 76/337/53). . . . RR Grinker analyses *Psychosomatic Research* (WW Norton, NY, 53, 208pp, $3.5). . . . C Hirsch & Co find age alterations in collagen polysaccharide ratio with decreased water content (*Acta Orthpaed Scand* 22/175/53). . . . G Jasmin & A Robert edit *Symposium on Mechanism of Inflammation* (*Acta*, Montreal, 53, 322pp, $8.8). . . . Our DW Micks & AA Benedict identify mosquitoes by infrared spectrophotometry (*Proc Soc Exp Biol Med* 84/12/53). . . . TM Newcomb suggest theory of communicative acts (*Psycho Rev* 60/393/53). . . . G Wald gives molecular basis of visual excitation (*Amer Sci* 42/73/54). . . . EG Wever & M Lawrence de-

scribe *Physiological Acoustics* (Princeton Univ Press, Princeton, NJ, 53, 540pp, $10).

FEBRUARY, 1954

CLINICAL: HB Atlee discusses *Chronic Iliac Pain in Women* (CC Thomas, Springfield, Ill, 54, 65pp, $2.5). . . . AL Barach & GJ Beck recommend head down position for relief in pulmonary empysema (*Am J Med* 16/55/54). . . . FC Combs describes *Coal Tar & Cutaneous Carcinogenesis in Industry* (CC Thomas, Springfield, Ill, 54, 75pp, $2.5). . . . ME DeBakey analyses thrombo-embolism (*SGO Internat Abs Surg* 98/1–27/54). . . . DD DeWeese offers *Dizziness: An Evaluation & Classification* (CC Thomas, Springfield, Ill, 54, 80pp, $2.5). . . . DL Fry & Co. review mechanisms of pulmonary ventilation in health & in emphysema (*Am J Med* 16/80/54). . . . MR Harrower & R Herrmann suggest *Psychological Factors in the Care of Patients with Multiple Sclerosis: For Use of Physicians* (National Multiple Sclerosis Soc, NY, 53, 32pp). . . . CP Rhoads discusses rational cancer chemotherapy (*Science* 119/77/Jan 15/54). . . . FE Russell reviews treatment of sting-ray injuries (*Am J Med Sci* 226/611/53). . . . WC Stadie surveys insulin action (*Physiol Rev.* 34/53/54). . . . JC White discusses pain conduction by afferent paths in cord & visceral nerves (*Arch Neurol Psychi* 71/1/54). . . . LL Wiesel & AS Barritt show p-amino benzoic acid aids cortisone therapy by delaying cortisone destruction (*Am J Med Sci* 227/74, 80/54). . . . F Wyss & J Regli analyse problem of cardiac dyspnoea (*Schw Med Wchnschr* 83/1175/Dec 5/53).

PRECLINICAL: HB Chase reviews hair growth (*Physiol Rev* 34/113/54). . . . RJ Dubos & JG Hirsch note thymus-peptide inhibition of mycobacteria (*J Exp Med* 99/55,65,79/54). . . . JM Garfunkel & Co show direct relation between oxygen consumption & cerebral function (*J Pediat* 44/64/54). . . . B Grad & Co find eosinopenic test satisfactory for assay of adrenal cortical steroid activity (*Proc Soc Exp Biol Med* 84/1/53). . . . D Granaat explores spleen in regulation of arterial blood pressure (*J Physiol* 122/209/53). . . . AG Hills & Co discuss adrenal cortical regulation of water & electrolyte distribution in humans (*J Clin Invest* 32/1236/53). . . . J Kieler discusses influence of amino acids on mitotic activity in fibroplast culture (*Acta Path Microbiol Scand* 33/337,350,361/53). . . . E Kovacs describes nucleases in cerebrospinal fluid (*Canad J Med Sci* 31/437/53). . . . EK Marshall & WF Fritz find alcohol in low conc in blood disappears at rate proportional to amount present, but at high conc at rate independent of amount (*J Pharmacol* 109/431/53). . . . E Mohme-Lundholm shows relaxing effect of adrenaline on smooth muscle is due to lactic acid formation (*Acta Physiol Scand* 29, Suppl 108, 53, 61pp). . . . K Neumann & G Koch demonstrate localization of succinic dehydrogenase by tetrazol purple (*Hoppe-Syeler's Ztschr Physiol Chem*, K Thomas Festschrift, 295/35/53). . . . MK Paasonen & TE Peltmen note increased tolerance to epinephrin in pregnancy (*Ann Med Exp Biol Fenn* 31/275/53). . . . NT Spratt Jr notes physiological mechanisms in development (*Physiol Rev.* 34/1/54). . . . R Stampfli describes saltatory conduction in nerve (*ibid* p 101). . . . JH Stover Jr & Co indicate botulinum

toxin inhibits acetylcholine release at motor end plates (*Proc Soc Exp Biol Med* 84/146/53). . . . MK Streifeld & MS Saslaw describe skin test for Rheumatic activity in children (*ibid* p 628). . . . M Taubenhaus & Co note inhibiting effect of dibenamine & banthine on granulation, like cortisone (*ibid* p 646).

GENERAL: Sir Edward Appleton appeals for science for its own sake (*Science* 119/103/Jan 22/54). . . . DW Atchley brings healer & scientist together in ideal full-time clinical teacher (*Sat Rev* Jan 9/54). . . . Lee T'ao describes achievements of Chinese medicine in Ch'in & Han periods (221–119GC) (*Chinese Med J* 71/380/53). . . . VF Lenzen discusses *Causality in Natural Science* (CC Thomas, Springfield, Ill, 54, 121pp, $3). . . . RR Loehrich offers remarkable philosophical analysis of semantics, values, dreams, psychosomatic ills, & interpersonal relations (*Oneirics & Psychosomatics: An Introductory Treatise Concerning a New Theory of Psychoanalysis, Its Logic & Methodology*, Compass Press, McHenry, Ill, 171pp). . . . EO Lippmann offers *Beitrage zur Geschichte der Naturwissenschaften und der Technik* (Verlag Chemie, Weinheim, 53, 2 vols, 380, 369pp, DM 45). . . . JC Moloney notes paradox of Japanese psychoanalysis where all individuals are merely part of the state (*Internat J Psychoanal* 34/291/53). . . . GK Plockmann edits *The Resources of Leonardo da Vinci* (Southern Illinois Univ, Carbondale, 53, 39pp). . . . T Reik offers *The Haunting Melody: Psychoanalytic Experiences in Life & Music* (Farrar Straus, NY, 53, 376pp, $4). . . . EA Underwood edits *Science, Medicine & History: Essays on the Evolution of Scientific Thought & Medical Practice, Written in Honour of Charles Singer* (Oxford Univ. Press, London, 54, 2 cols, 564 & 646pp, $45).